The effects of yarrow (*Achillea millefolium*) and herbal supplements on growth performance and nutrient utilisation of broiler chickens

Thesis

THE EFFECTS OF YARROW (*Achillea millefolium*) AND HERBAL SUPPLEMENTS ON GROWTH PERFORMANCE AND NUTRIENT UTILISATION OF BROILER CHICKENS

MICHELLE RHIANNON LEWIS

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ABSTRACT

The use of antimicrobial growth promoters in European poultry production will be banned in 2006, which has made it economically viable to investigate the use of other acceptable compounds, such as botanical products. Botanical products have a wide range of known pharmacological effects which could be exploited, for example carvacrol, found in oregano, has antibacterial properties.

Six experiments investigated the effects of botanical products on broiler growth performance. Two experiments were carried out to identify potentially useful products from a choice of six (garlic – *Allium sativum*, horseradish – *Amoracia rusticana*, juniper – *Juniper communis*, milk thistle – *Silybum marianum*, oregano – *Origanum vulgare* and yarrow – *Achillea millefolium*). Garlic powder and yarrow supplementation improved FCE ($P<0.05$) by 12 and 13% relative to controls in the second experiment.

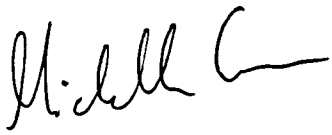
Two further experiments were floor-pen studies to examine the effects of both garlic and yarrow on growth performance. No performance benefits were associated with garlic supplementation ($P>0.05$). Feeding yarrow supplemented diets resulted in improved weight gain ($P<0.05$) during the grower phase but no effects on caecal microflora were detected ($P>0.05$).

Yarrow contains 'bitter' sesquiterpene compounds (cadinene and germacrene), which may stimulate digestive enzyme production, so the fifth experiment examined the effects of dietary yarrow on digestive enzyme activity and nutrient availability of birds fed control and low nutrient density basal diets. Yarrow supplementation increased intestinal lipase activity and improved diet AME in birds fed high nutrient density diets but not in birds fed low nutrient density diets (yarrow x diet density interaction $P<0.05$). Therefore, the final experiment examined the interactions between yarrow supplementation and fat source. Yarrow supplementation improved growth performance ($P<0.01$) of birds fed saturated fat sources with a concomitant increase in gizzard bile acid concentrations ($P<0.01$), which may indicate increased gastrointestinal reflux.

DECLARATION

This thesis was composed by the author and is a record of work carried out by her in an original line of research. All sources of information are shown in the text and listed in the references; all help given by others is indicated in the acknowledgements.

None of this work has been presented in any previous application for a degree.



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This thesis is dedicated to the memory of Mordecai Lewis.

PUBLISHED WORK

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LIST OF ABBREVIATIONS

AGP	Antimicrobial Growth Promoter
ANOVA	Analysis of variance
AME	Apparent metabolisable energy
BTEE	Benzoyl-L-tyrosine ethyl ester
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
GRAS	Generally recognised as safe
MRD	Maximum recovery diluent
NE	Necrotic enteritis
NSP	Non-starch polysaccharide
SEM	Standard error of the mean
TAME	*p*-toluenesulphonyl-L-arginine methyl ester

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1. INTRODUCTION

Continued advances in broiler chicken feeds are an important component of the success and efficiency of today's UK broiler industry. Improvements in broiler diet formulations have not only been achieved through better supply and balance of nutrients, but also in the development and use of in-feed antibiotic growth promoters (AGP). However, AGP are currently under intense scrutiny amid fears that their use may result in antibiotic residues in animal tissue (HMSO, 1998). Also, there are concerns that long term use may select for the survival of resistant bacteria and strains, which may then be transferred to other bacteria, thus making them resistant (Aarestrup, 1999).

In response to the possible detrimental effects on human health, the European Union has decided to phase out the usage of AGP. Effective as of July 1999 the European Union banned all but four drug-related compounds for routine use in animal feed, the remaining products being avilamycin, flavophospholipidol, monensin and salinomycin (Johnston, 2001). It is widely speculated that these four AGP will be prohibited by 2006 at the latest and that no new antibiotic feed additives will be authorised (McCartney, 2002; Pearson, 2002). Following a voluntary AGP ban in Sweden in 1986, an increase in problems such as wet litter and an increased incidence of diseases such as necrotic enteritis have been observed (Wierup, 2001). These problems result in loss of production efficiency, and ultimately profitability. It is imperative that the UK industry remains competitive against imported poultry meat so there is an urgent need to develop reliable alternatives to traditional AGP.

It is clear that AGP have been heavily relied upon for profitable broiler production, and that there will be a substantial gap in the market when their use is banned. Suggestions for

increasing the performance of poultry in the absence of AGP include a variety of specific nutritional supplementation options, with organic acids (acidifiers), enzymes, probiotics and botanical products and essential oils generating the most interest. The scope of this work is focussed on the potential use of botanical products and essential oils as natural growth promotants.

Botanical products and essential oils contain a wide range of pharmocological properties, which may be exploited in order to improve broiler performance in the absence of AGP. Indeed, there is a myriad of commercially available products (e.g. Crina® Poultry, Akzo Nobel, Switzerland; Orego-Stim®, Meriden Animal Health, UK; Apex® Poultry, BFI Innovations, UK), but little published evidence to support manufacturers' claims or indicate mode of action. Problems with botanical extracts include the complexity and lack of knowledge of natural plant chemistry, lack of uniformity between samples, synergistic responses to other components of the diet *in vivo*, and residue safety. Many herbal compounds have been shown to demonstrate antibacterial activities *in vitro* but the application of these activities may be dependent on the availability and synergy of these compounds *in vivo*. Studies involving monogastric animals have shown varied reports on the beneficial effects of plant extracts and essential oils; and very few reported experiments include analysis of the active ingredients in the botanical extracts tested.

In order to harness the potential benefits of botanical products and promote them as viable alternatives to AGP, their quality, safety and efficacy must be investigated. The specific objectives of the current study were to examine the efficacy of selected botanical extracts on the efficiency of growth and nutrient availability of growing broiler chickens and elucidate probable mode of action.

2. LITERATURE REVIEW

2.1 Antimicrobials in poultry feed

Antibacterial growth promoters (AGP) have been added to poultry feed at subtherapeutic levels since 1946 when Moore and workers first reported their positive effects on growth performance (Moore *et al.*, 1946). Most antibacterials are isolated from naturally growing fungi and bacteria (antibiotics), although some are produced synthetically (Walton, 2001). Antimicrobials act either bactericidally (i.e. kill bacteria on contact e.g. penicillin) or bacteriostatically (i.e. prevent bacteria from multiplying e.g. tetracyclines). There is a third group of antimicrobials, ionophores, which also control protozoal growth (Thomke and Elwinger, 1998a).

AGP have been integral in the development of today's broiler industry, making it possible to improve animal health conditions as well as increasing rearing intensity, whilst simultaneously lowering food production costs. Broilers fed AGP have increased weight gain, muscle yield and feed conversion (Rosen, 1995). From a nutritional stance, AGP are believed to inhibit growth of intestinal bacteria with growth-depressing properties for the chicken competing with the host for available nutrients (Muramatsu, 1994).

Despite the benefits to the agricultural industry and domestic animals, there is fierce debate concerning the practice of feeding AGP because of the possible risks to human health. Some AGP are closely related to antibiotics used in the treatment of bacterial infections in humans (Gustafson and Bowen, 1997), and there are concerns that there is a potential for bacterial

resistance to occur, resulting in reduced antibiotic efficacy in humans (Greko, 2001; Witte, 2001; Salisbury *et al.*, 2002). Avoparcin was the first AGP to be removed in the EU, as it was found to select for Vancomycin-resistant *Enterococci* (Greko, 2001). Bager and workers (1997) undertook a study of 22 pig herds, and compared them on the basis of avoparcin use and occurrence of Vancomycin-resistant *Enterococci* in faecal samples. They observed Vancomycin-resistant *Enterococci* in 66 and 20% (P=0.043) of herds fed and not fed avoparcin respectively. However, Follet (2000) cites a study by Casewell (1998) who examined the antibiotic resistance profiles of human and poultry Vancomycin-resistant *Enterococci* isolates. Although the two strains possessed many common features, they were found to be two statistically distinct populations. Casewell concluded that the evidence implicating a transfer of resistance from animals to humans was lacking, and that banning AGP was "most unlikely" to have any impact on resistance problems seen in the human health sector.

Virginiamycin has been shown to induce bacterial cross-resistance to Synercid (dalfopristin and quinupristin), an antibiotic developed for the treatment of infections caused by gram positive bacteria in human medicine (Aarestrup, 2000). Several studies report *Escherischia coli* resistance to antibiotics, as up to 86% of the *E. coli* isolated from UK pigs have proven to be resistant to tetracycline, and 53 and 42% to sulphamethoxazole/trimethoprim and ampicillin respectively (Blake *et al.*, 2003).

In response to the possible detrimental effects of AGP on human health, the European Union decided to phase out their usage. Effective as of July 1999, the EU banned all but four AGP for routine use in broiler feed, the remaining products being avilamycin, flavophospholipidol, monensin and salinomycin (McCartney, 2002). It is widely speculated that these four AGP

will be prohibited by 2006 at the latest, and that no similar new products will be authorised (McCartney, 2002). Available data show that AGP usage in EU animal production is declining (Figure 2.1). According to the Veterinary Medicines Directorate (VMD), AGP accounted for approximately 5% of the total amount of antimicrobials sold in the UK in 2000. There has been a sharp decline in UK AGP usage over the last decade, with sales falling by 71% since 1993 (VMD, 2002).

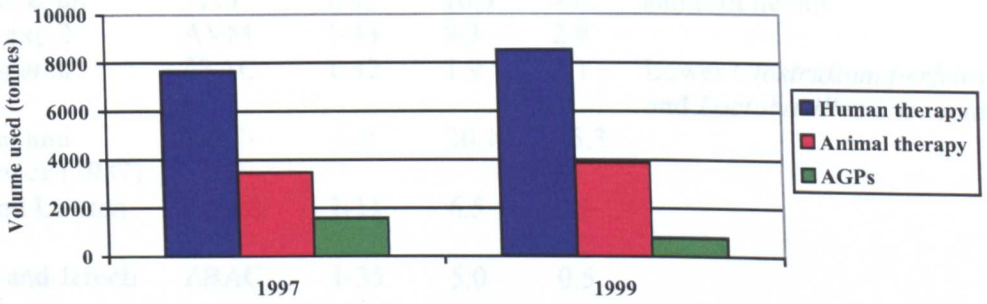

Figure 2.1. Antibiotic usage in the EU (1997-1999)

Source: European Federation of Animal Health, 2001.

2.1.1 Effectiveness of AGP

Antibacterials as nutrition improvers are used to reduce production costs by improving broiler growth performance and health status. Their benefits are well documented, but the responses are highly variable (table 2.1). Responses appear to be relatively greater when broilers are raised in unfavourable conditions, such as high stocking densities (Dafwang *et al.*, 1987), poor hygiene status and high pressure of infectious disease (Rosen, 1995; Elwinger *et al.*, 2000). In addition, heavy weight broilers respond better than light weight broilers (Rosen, 1995), and responses are greater in young broilers than older broilers (Armstrong, 1986; Rosen, 1995).

Table 2.1. Growth responses to various AGP relative to unsupplemented control diets

Source	AGP	Age interval, days	Effect on (%) GP	FCR	Comment
Broz *et al.* (1994)[a]	VIM	1-35	1.8	3.4	
Dafwang *et al.* (1987)	PEN	1-28	9.2	4.3	
Elwinger *et al.* (1998): exp 1	AVP	1-45	6.5	4.6	Lower *Clostridium perfringens* counts; improved litter quality and foot health
	AVM	1-45	4.4	4.1	
Elwinger *et al.* (1998): exp 2	AVP	1-43	10.3	4.0	
	AVM	1-43	8.3	2.9	
Engberg *et al.* (1999)	ZBAC	1-42	1.9	3.1	Lower *Clostridium perfringens* and *Lactobacillus* spp. counts
Feighner and Dashkevicz (1987)	BAC-M	1-9	20.1	15.3	
Stutz and Lawton, (1984)	ZBAC	1-11	6.5	5.5	
Schurz and Jeroch (1994)[a]	ZBAC	1-35	5.0	0.5	
Waldenstedt *et al.* (1999)	VIM	1-36	13.7	0	Lower *Clostridium perfringens* counts

[a] cited in Thomke and Elwinger, 1998a; AVM, avilamycin; AVP, avoparcin; BAC-M, bacitracin methylenedisalicyclic acid; PEN, penicillin; VIM, virginiamycin; ZBAC, zinc bacitracin

The overall responses of chickens to the promotants listed in table 2.1 with regards to growth rate and feed efficiency in comparison with chickens fed unsupplemented control diets are in the region of 7.9 and 4.4% for growth rate and feed efficiency respectively. A review by Armstrong (1986) concluded that the use of AGP improves weight gain by 5-6% and feed conversion efficiency by 3-4%, with the most pronounced effects observed in young animals. Rosen (1995) reviewed over a thousand tests relating to AGP feeding in broilers, and quoted a lower figure of 2% improvement in weight gains. However, this lower estimate of gain may be because he included experiments where zero and negative responses to AGP were noted.

In addition to their growth promoting properties, AGP have also been shown to improve litter quality through a reduction of excreta moisture content (Elwinger *et al.*, 1996). Not only is this

advantageous in terms of bird health and hygiene, but also in terms of reducing ammonia formation, which improves bird environment and reduces environmental air pollution (Elwinger and Svensson, 1996).

2.1.2 Mode of Action

The mode of action of AGP in pigs and poultry has been the subject of much scientific investigation, yet the mechanisms of growth promotion by these products are still speculative. However, the primary mode of action implicates a moderation of the gut flora, since AGP do not improve the growth of germ-free broilers (Forbes and Park, 1959; Coates *et al.*, 1963). Evidence suggests that AGP inhibit the growth of intestinal bacteria with growth depressing properties (Fuller *et al.*, 1979; Engberg *et al.*, 2000). Gastrointestinal microbial populations play a complex role in nutrition and growth that is poorly understood despite extensive research. However, it is clear that microflora have several deleterious effects on growth and efficiency: competing with the host for nutrients in the digestive tract (Muramatsu *et al.*, 1994); increasing prevalence of disease (particularly necrotic enteritis) (Stutz and Lawton, 1984; Hruby and Remus, 2001); lowering digestive efficiency by degrading digestive enzymes and bile salts (Feighner and Dashkevic, 1987; Engberg *et al.*, 2000); and increasing the size of the intestinal tract and thus the energy requirements for gut maintenance (Stutz and Lawton, 1984). The negative impact of microflora on bird performance is demonstrated by the work of Murumatsu *et al.* (1994) who showed that germ-free birds grew faster than their conventional counterparts, even though they captured significantly less ($P<0.01$) energy from the diet (10.8 *vs.* 10.3 MJ $kgDM^{-1}$ AME for conventional and germ-free chicks respectively; $P<0.01$). The discrepancy in AME values is a consequence of microflora extracting a significant amount of energy from the diet, which in this experiment amounted to approximately 10% of the diet.

Boorman (1987) demonstrated that AME content of AGP supplemented diets is increased by 5% compared to unsupplemented control diets, implicating a beneficial moderation of gut microbial populations. Feighner and Dashkevicz (1987) showed that conventional chickens fed AGP supplemented diets grow and exhibit feed efficiencies approaching those achieved by germ-free chickens.

Antimicrobial growth promoters also decrease the fermentation of carbohydrates and the decomposition of bile salts (Feighner and Dashkevicz, 1987; Engberg *et al.*, 2000). These changes increase the availability of nutrients and energy to the animal host (Eyssen, 1962; Engberg *et al.*, 2000), and decrease the concentration of toxic molecules like ammonia or amines in the gut, leading to a reduced turnover in the gut epithelium (Coates *et al.*, 1963).

Aside from the influences on the intestinal microflora, results from experimental work by Engberg *et al.* (2000) indicate that zinc-bacitracin stimulates activity of the pancreatic enzymes amylase, chymotrypsin and lipase. It has also been suggested that AGP change nutrient absorption efficiency: March and Biely (1967) claim that this is a result of intestinal wall thinning which is thought to facilitate nutrient absorption. Numerous others workers have demonstrated that AGP reduce intestinal wall thickness (Jukes *et al.*, 1956, Stutz *et al.*, 1983a, Stutz *et al.*, 1983b, Stutz and Lawton, 1984) with a concomitant positive effect on growth performance.

2.1.3 Implications of AGP Ban

With so many variables affecting performance in broiler production it is difficult to determine if AGP withdrawal will have any influence on growth performance. However, Pearson (2002) studied the EPEF (European Production Efficiency Factor) values for three British companies (December 1997 – May 2002), all of whom had removed AGP in late 1999/early 2000. The average results of the three companies demonstrate that performance improvements have not kept pace with what would be expected with normal rates of annual genetic improvement. This was attributed to poorer feed conversion ratio (FCR) associated with AGP removal.

Several European countries have voluntarily banned the use of AGP already: Sweden in 1986 (Wierup, 2001b), Norway in 1998 (Wierup, 2001a) and Denmark in 1998 (Knudsen, 2001). A higher incidence of necrotic enteritis (NE) has been associated with AGP removal in Norway (Schaller, 1998) and Sweden (Wierup, 2001b). NE is caused by the proliferation of *Clostridium perfringens* Type A or C in the small intestine (Porter, 1998; Lovland and Kaldusdahl, 2001) which produces alpha-toxins resulting in a loss of gut wall integrity (Fickens and Wages, 1997), wet litter, diarrhoea and liver lesions (Morrow, 2001). The main economic losses associated with NE and similar diseases are not from mortality, as birds usually recover, but from a marked reduction in production efficiency (Morrow, 2001). Increases in problems such as wet litter have been observed in Sweden since the ban, with a concomitant increase in carcass downgrades at slaughter (Wierup, 2001b). Inborr (2001) also documents 4- to 5- fold increases in carcass downgrades as a result of liver lesions in Sweden following the AGP ban.

However, a study of data collected from 6,815 Danish broiler flocks (November 1995 – June 1999) showed no evidence of decreased productivity (kg broiler produced/m^2) or increased mortality following AGP removal, although an increase in feed consumption (0.016kg feed/kg broiler) was reported (Emborg *et al.*, 2000). Assuming an annual crop of 660 million birds in the UK, this equates to approximately 26,400 tonnes extra feed at an estimated cost (assuming broiler feed costs of £142 per tonne (Nix, 2003)) of £3.75 million annually.

Garland (1995) calculated that the use of AGP gives and extra 23,100 tonnes of bird liveweight with a saving of 91,000 tonnes of feed annually in the UK. He values the financial advantage of AGP inclusion at 0.035 ECU (2.885 pence) per bird, which equates to a gross financial saving of 23 million ECU (approximately £19 million) annually in the UK alone.

2.1.4 Summary

AGP have been used for many years by the poultry industry and have proved to be an effective tool in enhancing animal health status, uniformity and production efficiency. The consequences of their removal are many: possible reductions in growth performance, increased incidence of disease, particularly necrotic enteritis, increased health and welfare problems, and ultimately, reduced profitability. Their removal from diets will therefore be a difficult obstacle to overcome, particularly if EU broiler production is to remain competitive with that of the rest of the world, where such products are likely to remain in use.

2.2 Alternatives to AGP

A number of strategies are available for increasing the performance of poultry in the absence of AGP (Collett and Dawson, 2002). Many of these strategies are based on management techniques, but a number of specific nutritional supplementation strategies are also available, with organic acids (acidifiers), enzymes, probiotics and plant extracts and essential oils generating the most interest as possible AGP replacements.

2.2.1 Organic Acids (Acidifiers)

Organic acids commonly employed in poultry nutrition include acetic, citric, formic, fumaric and lactic acids (Dibner and Buttin, 2002). Since these acids are fully metabolisable, they are utilised either by the bird or by the micro-organisms in the gastrointestinal tract, which eliminates the need for 'withdrawal' diets (Adams, 1999). Dietary acidification has been extensively researched in pigs, but inconsistent performance effects have been observed (Thomke and Elwinger, 1998c). In general, improvements in feed efficiency tend to be more consistent than body weight gains (Piva and Rossi, 1999), and positive effects are most pronounced in weanling pigs (Gabert and Sauer, 1994; Roth and Kirchgessner, 1998).

Acidification of poultry feeds also produces variable growth performance effects. Patten and Waldroup (1988) demonstrated that addition of 0.5 or 1.0% fumaric acid significantly ($P<0.05$) improved body weights of broilers fed to 21 days but did not influence feed utilisation. This was corroborated by Skinner *et al.* (1991) who found that addition of 0.5% fumaric acid significantly ($P<0.05$) improved 49 day body weight of female and male broiler chickens with no effect on feed utilisation. However, other workers have failed to demonstrate

any positive production benefits associated with dietary acidification (Alp *et al.*, 1999; Waldroup *et al.*, 1995). In addition, Cave (1984) reported that supplementation of chick diets with proprionic acid resulted in depressed feed intakes and weight gains.

It is thought that dietary acidification inhibits pathogenic enteric bacteria, and thus promotes a better microbial balance in the gastrointestinal tract (Hillman, 2001). The acids lower the pH in the gut, which directly affects optimum conditions for different classes of bacteria. Approximate pH ranges for microbial growth vary according to bacterial species, with *Escherichia coli* and most *Salmonella* species having an optimum pH of 6.0-8.0 and 6.8-7.2 respectively, and *Lactobacillus* species favouring lower levels of 5.4-6.4 (Hyden, 2000). Thus lowering gut pH favours the proliferation of beneficial bacteria and reduces pathogenic strains. Lower concentrations of pathogenic bacteria reduce disease incidence, but also result in decreased gut wall thickness and increased villi length leading to improved feed conversion (Doyle, 2001). They may also help by solubilising feed ingredients and improving digestion and absorption of feed ingredients (Thomke and Elwinger, 1998c).

2.2.2 Enzymes

Recent market research indicates that, on a world-wide basis, as much as 60-70% of wheat and barley based poultry feeds are enzyme supplemented to overcome the antinutritive factors associated with feeding these cereals (Cleophas *et al.*, 1995; Partridge and Wyatt, 1995). However, response to enzyme supplementation is variable and the mechanisms of action have not been fully elucidated (Thomke and Elwinger, 1998c).

Barley, wheat, rice and rye contain anti-nutritive factors that impede digestion and absorption when fed to poultry, resulting in negative effects on growth and FCR (Friesen *et al.*, 1992; Marquardt *et al.*, 1994; Choct *et al.*, 1995). Dietary addition of NSP-degrading enzymes has been shown to reduce intestinal viscosity and increase nutrient digestibility, particularly fat digestibility, in chicks fed diets containing these cereal sources (Hesselman *et al.*, 1982; Annison, 1992; Friesen *et al.*, 1992; Marquardt *et al.*, 1994; Choct *et al.*, 1995; Steenfeldt *et al.*, 1998a; Steenfeldt *et al.*, 1998b; Zanella *et al.*, 1999). Hock *et al.* (1997) demonstrated that the performance benefits seen in wheat fed broilers with enzyme supplementation were comparable to those seen in birds fed AGP, but that there was no additive effect seen by feeding enzymes and AGP in tandem.

Other investigators have failed to detect positive effects of enzyme supplementation on broiler growth performance (Alloui *et al.*, 1994; Annison *et al.*, 1996; Douglas *et al.*, 2000), particularly when high quality fat sources are used (Langhout *et al.*, 1997).

The pronounced increase in fat digestion following enzyme supplementation suggests that the activity of intestinal bacteria capable of bile acid conjugation is inhibited. Enzyme effects are more pronounced in diets containing animal fats than plant oils (Smulikowska and Mieczkowska, 1996; Daenicke *et al.*, 1997a; Daenicke *et al.*, 2000; Preston *et al.*, 2001), which could relate to the fact that higher gut viscosity is observed in birds when fats containing higher degrees of saturation are fed (Daenicke *et al.*, 1997b; Daenicke *et al.*, 2000). Also, increasing nutrient digestibility reduces the amount of substrates available for bacterial growth and fermentation (Bedford, 2000; Engberg and Petersen, 2001), and the associated detrimental effects on growth depression described earlier in this review. Published work suggests that the benefits of enzyme supplementation are greater in younger chicks (Steenfeldt

et al., 1998a; Steenfeldt *et al.*, 1998b), probably as a result of the greater antinutritional effects of wheat seen in younger chicks (Veldman and Vahl, 1994).

2.2.3 Probiotics

Fuller (1992) defined a probiotic as a "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". Some 42 different probiotic microorganisms are currently considered GRAS (Generally Recognised As Safe) (Stavric and Kornegay, 1995): 19 of these are authorised in the EU, 7 of which are available for poultry (Simon and Jadamus, 2002). Organisms authorised for poultry belong to the bacterial genera *Lactobacillus, Streptococcus, Enterococcus, Pediococcus* and *Bacillus,* with microscopic fungi (*Saccharomyces* yeasts) also available (Stavric and Kornegay, 1995; Thomke and Elwinger, 1998c; Guillot, 2001).

Jin and workers (1998a) investigated the effects of feeding a *Lactobacillus* culture to growing broiler chickens up to 42 days of age. Body weights and feed to gain ratios (at 21 and 42 days of age) were improved significantly ($P<0.05$) in comparison to chicks fed unsupplemented control diets. Numerous other studies have reported the beneficial effects of feeding *Lactobacillus* cultures (Tortuero, 1973; Dilworth and Day, 1978; Jin *et al.*, 1998b; Watkins *et al.*, 1982), and *Lactobacillus* cultures have also been found to reduce caecal coliforms (Jin *et* al., 1998b), lower abdominal fat deposition (Kalavathy *et* al., 2003), lower serum lipid concentrations (Kalavathy *et* al., 2003) and enhance egg production and quality (Nahashon *et al.*, 1994). In contrast, several workers have failed to demonstrate significant performance effects as a result of probiotic supplementation (Watkins and Kratzer, 1983; Watkins and Kratzer, 1984; Maiolino *et al.*, 1992; Yeo and Kim, 1997; Simon and Jadamus, 2001).

Several studies have focussed on comparing probiotics and traditional AGP against unsupplemented controls with varying results. Work by Guillot (2001) highlighted that *Bacillus* strains improved chick growth by 1.5% relative to controls, but that the improvements seen when an AGP was fed were higher (2.1% higher than controls). However, in the same experiment, another probiotic (*Enterococcus* spp.) depressed growth by 1.7% relative to controls. Zulkifi and workers (2001) demonstrated that chicks fed a *Lactobacillus* probiotic showed higher ($P<0.05$) weight gains and feed intakes relative to chicks fed control diets and AGP supplemented diets up to 42 days, but that the FCE of these birds was lower ($P<0.05$) than birds fed the other treatment diets. Conversely, Onifade *et al.* (1999) reported that birds fed a yeast derived probiotic (*Saccharomyces cerevisiae*) showed higher weight gains ($P<0.05$) than their conspecifics, and decreased FCR relative to control fed ($P<0.001$) and AGP ($P<0.05$) fed birds. Yeast supplementation has also been shown to ameliorate the effects of immunological challenge in pigs (Auclair, 2001).

The mechanisms of action for probiotics are as yet unclear, but several theses are speculated: increased competition for adhesion receptors on the gut epithelium (competitive exclusion); competition with pathogenic bacteria for nutrients; production of antibacterial substances (e.g. lactic and acetic acids); stimulation of immunity; and reduction of intestinal pH through acid production (Vanbelle *et al.*, 1990; Fuller, 1992; Thomke and Elwinger, 1998c; Collins and Gibson, 1999; Guillot, 2001; Simon and Jadamus, 2001).

2.2.4 Botanical Products

Bioactive principles from plants are essentially secondary metabolites. Secondary metabolites differ from primary metabolites (i.e. carbohydrates, proteins) in that their distribution is limited (Greathead, 2003). They are generally produced by specific plants, or groups of plants, for example phenols found in the *Labiatae* family of plants (Kamel, 2001a). It is thought that the diversity of secondary metabolites has evolved to encourage plant survival through protection from pathogens, and by attracting beneficial organisms such as pollinators (Harborne *et al.*, 1999; Greathead, 2003). Plant secondary metabolites provide a wide selection of biologically active compounds (Deans and Svoboda, 1990), and a varied range of pharmaceutical products derived from plants have been exploited since antiquity (Dorman and Deans, 2000), while natural antimicrobials, flavourings and antioxidants have played a role in food preservation (Fowler, 1980). However, since the advent of antibiotics in the 1950s, the use of plants and their derivatives as antimicrobials has been virtually non-existent (Cowan, 1999).

Published experimental work on the influence of herbs and their extracts on broiler performance has increased recently. However, the majority of available literature reports 'production type' experiments with little focus on mechanisms of action, or actual chemical analysis of products used. Also, reported experiments tend to use herbal products of proprietary origin whose exact herbal composition is subject to secrecy.

2.3 Plants and their Secondary Metabolites

Herbs and spices consist of dried leaves (mint, sage), flowers (borage, savory), buds (clove), fruits (coriander, pepper, pimento), seeds (fenugreek, liquorice, turmeric, ginger) and parts of fruits (aril of mace) (Stanley and Svoboda, 1990). They are reported to have different beneficial pharmacological effects, with many variant mechanisms of action (table 2.2). The beneficial effects of herbs come from one or more types of bioactive components, often acting synergistically (Macrae *et al.*, 1993; Cowan, 1999; Wills *et al.*, 2000). These chemically distinct, but often overlapping, classes of bioactive components are mainly terpenoids (such as sesquiterpenes and saponins), phenols (such as tannins) and their glycosides (flavonoids, glucosinolates), and mucilages, with essential oils often containing several of these classes (Bruneton, 1995; Tyler *et al.*, 1998; Harborne *et al.*, 1999). In order to evaluate the efficacy of plants and their secondary metabolites in broiler nutrition, these groups of bioactive components must be reviewed in order to conjecture potential benefits and elucidate mechanisms of action.

Table 2.2 Summary table representing potentially useful plant extracts, their utilised parts, main active components and reported properties

Plant	Utilised part	Main components	Reported properties
Anise	Fruit	Anethol	Digestion stimulant
Bay laurel	Leaf	Cineol	Appetite and digestion stimulant; antibacterial
Capsicum	Fruit	Capsaicin	Anti—inflammatory; stimulant; tonic
Cardamom	Seed	Cineol	Appetite and digestion stimulant
Celery	Fruit, leaf	Phtalides	Appetite and digestion stimulant
Cinnamon	Bark	Cinnamaldehyde	Appetite and digestion stimulant; antibacterial
Clove	Cloves	Eugenol	Appetite and digestion stimulant; antibacterial
Coriander	Leaf, seed	Linalool	Digestion stimulant
Cumin	Seed	Cuminaldehyde	Digestive stimulant
Fenugreek	Seed	Trigonelline	Appetite stimulant
Garlic	Bulb	Allicin	Digestive stimulant; antibacterial
Ginger	Rhizome	Zingerone	Gastric stimulant
Horseradish	Root	Allyl isothiocyanate	Digestion stimulant
Juniper	Berry	Not known	Appetite and digestion stimulant
Milk thistle	Fruit	Silymarin	Hepatoprotectant
Mustard	Seed	Allyl isothiocyanate	Digestion stimulant
Nutmeg	Seed	Sabinene	Digestion stimulant; antidiarrhoeic
Oregano	All	Thymol; carvacrol	Appetite and digestion enhancing; antioxidant; antibacterial
Parsley	Leaf	Apiol	Appetite and digestion stimulant; antibacterial
Pepper	Fruit	Piperine	Digestive stimulant
Peppermint	Leaf	Menthol	Appetite and digestion stimulant; antibacterial
Rosemary	Leaf	Cineol	Digestion stimulant; antibacterial; antioxidant
Sage	Leaf	Cineol	Digestion stimulant; antibacterial
Thyme	All	Thymol; carvacrol	Appetite and digestion enhancing; antioxidant; antibacterial
Yarrow	All	Not known	Appetite and digestive stimulant; antibacterial; anti-inflammatory

Bruneton, 1995 and 1999; Kamel, 2001a, b and c; McCartney, 2002; Samarasinghe and Wenk, 2002

2.3.1 Essential Oils

Essential or volatile oils may be defined as "odoriferous bodies of an oily nature obtained from plant leaves, flowers, fruit, seed, wood, resin, bark or roots" (Thomas, 2000) and are a complex mixture of various compounds (Leung and Foster, 1996). They are obtained generally in liquid, semisolid or solid form, and usually can be steam distilled and solvent extracted (Macrae *et al.*, 1993). Essential oils do not dissolve in water or dissolve only very poorly, evaporate at room temperature without residue, and often have characteristic strong odours or tastes (Bruneton, 1995).

Essential oils are the products of a plant's metabolic processes and as such are subject to seasonal and climatic changes, crop husbandry, harvesting, processing and storage variables (Macrae *et al.*, 1993). Essential oils are produced in various secretory tissues specific to particular plants: oil and oleoresin cells (ginger, pepper), secretory glands (bay, cloves), secretory ducts (tarragon, angelica) and glandular trichomes (sage, rosemary) (Bruneton, 1995).

Kohlert and workers (2000) reviewed the various pure components of essential oils commonly used in herbal medicine and summarised their absorption, metabolism and excretion. They concluded that essential oil constituents are quickly absorbed after administration (oral, pulmonary or dermal), and that most are either eliminated by the kidneys or exhaled as carbon dioxide. They also surmised that accumulation in body tissues is unlikely due to rapid metabolic conversion and short half lives.

2.3.2 Terpenoids

Terpenes

Terpenes can be subdivided into four catergories: monoterpenes, sesquiterpenes, diterpenes and triterpenes (Wagner and Wolff, 1977).

Monoterpenes are ubiquitous in the plant kingdom, but tend to accumulate in members of certain families, such as the *Juniperus* species (Bruneton, 1995). They are also widely distributed in the Labiatae family, for example in oregano and thyme (Baratta *et al.*, 1998a), and are found in yarrow (Cowan, 1999). Their primary functions in plants are attraction of pollinators to flowers, and protection from microbial infection (Harborne *et al.*, 1999).

Sesquiterpenes are widespread in higher plants (Harborne *et al.*, 1999), but are most abundant in the Compositae family (Rodriguez *et al.*, 1976). A sesquiterpene of particular interest in this work is chamazulene (Chamomiles; yarrow) which possesses anti-inflammatory (Rodriguez *et al.*, 1976; Picman, 1986; Chandler, 1989; Safayhi *et al.*, 1994; Chevallier, 1996), anti-bacterial (Rodriguez *et al.*, 1976; Chevallier, 1996) and anti-oxidant (Safayhi *et al.*, 1994; Rekka *et al*, 1996) properties. Preliminary research of anecdotal evidence indicates that yarrow (*Achillea millefolium*) possesses tonic (Mitch, 1990), digestive stimulating (Chandler, 1989) and digestive enhancing (McCartney, 2002) properties, and as such is a promising herb for inclusion in poultry diets.

Diterpenes are principally found in higher plants and fungi, but are of very restricted distribution (Harborne *et al.*, 1999) and are rarely found in essential oils (Wagner and Wolff, 1977).

Saponins

Saponins are a heterogenous group of water soluble triterpenoids which are widely distributed in the plant kingdom, (Macrae *et al.*, 1993) and are known for their bitter, astringent taste (Cowan, 1999). Feeding high levels of ingredients containing saponins has been shown to have negative effects on growth performance in poultry, for example Jack beans (Belmar *et al.*, 1999), sheanut cake (>25kg tonne^{-1}; Atuhene *et al.*, 1998) and quinoa (>25kg tonne^{-1}; Jacobsen *et al.*, 1997). However, at lower inclusion levels they may improve absorption of nutrients (McCartney, 2002). This may be as a result of their surfactant properties which are thought to condition the cell membranes and reduce surface tension leading to increased absorption of nutrients across cell membranes (Johnston *et al.*, 1981; Johnston *et al.*, 1982; Johnson *et al.*, 1986).

A major commercial source of saponins is *Yucca chidigera,* which grows in the arid Mexican desert (Cheeke, 1999). Extracts of *Yucca chidigera,* produced by drying and pulverising the stem, are reported to contain 1-1.5% active saponin (Johnston *et al.*, 1981; Johnston *et al.*, 1982). Cromwell and workers (1985) reported that diets containing *Y. schidigera* improved growth rate in weaner pigs, but Yen and Pond (1993) did not find any improvement in growth rate when they fed the same extract to weaner pigs. Another study (Gipp *et al.*, 1988) also failed to demonstrate beneficial effects of *Y. schidigera* extract in growing pigs, with or without AGP supplementation.

Yeo and Kim (1997) fed *Y. schidigera* extract (2kg tonne^{-1}) and did not observe any significant positive effects on broiler growth performance between 0 and 6 weeks, although performance attained by *Y. schidigera* supplemented birds was numerically superior to those fed unsupplemented control diets. However, Johnston and his team have demonstrated

consistent positive growth performance effects as a result of feeding *Y. schidigera* (63 mg kg^{-1}) to broiler chickens. Bird body weights attained by dietary yucca supplementation were 3.1 and 3.4% higher ($P<0.05$) at 28 and 51 days of age respectively relative to unsupplemented controls, with no effect on feed intake (Johnston *et al.*, 1981). Similar effects were seen when the same extract was fed with and without AGP supplementation (Johnston *et al.*, 1982), where birds fed diets supplemented with *Y. schidigera* and AGP in combination grew faster than those fed the AGP alone.

2.3.3 Phenolics and Polyphenols

Phenols

Phenols have strong antioxidant properties (Economou *et al.*, 1991). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting oxidising chain reactions. The antioxidant activity of phenolic compounds is mainly due to their redox properties which play an important role in neutralising free radicals and decomposing peroxides (Zheng and Wang, 2001). Plants belonging to the *Lamiaceae* family (e.g. oregano, thyme) tend to be high in phenols (Macrae, 1993). Economou *et al.* (1991) evaluated the antioxidant properties of extracts of oregano, dittany, thyme, marjoram, spearmint, lavender and basil when added to lard at 75°C. Oregano extract was found to be the most effective at stabilising lard, followed by thyme. Both contain *p*-cymene, thymol and carvacrol, that are all principles demonstrated to have strong antioxidant properties (Aeschbach *et al.*, 1994; Aruoma *et al.*, 1996; Baratta *et al.*, 1998b). Farag *et al.* (1991) suggested that the high antioxidant activity of thymol is due to the presence of phenolic hydroxide groups which act as hydrogen donors to the peroxy radicals produced during the first step of lipid peroxidation.

Flavonoids

Flavonoids are most abundant in the photosynthetic cells of higher plants (Havsteen, 1983). More than 500 flavonoids have been identified, both in the plant and animal kingdoms (Valenzeula and Garrido, 1994). For centuries, a number of different therapeutic and curative properties have been attributed to them and they are widely used in herbal medicine (Bito *et al.*, 2002). Flavonoids such as quercetin and taxifolin have been used as pharmacological principles for anti-inflammatory applications (Harborne *et al.*, 1999) and for the treatment of skin inflammatory diseases (Bito *et al.*, 2002) respectively. Anecdotal evidence suggests that they may have strong diuretic properties, enhance immune response, stimulate bile production (McCartney, 2002) and assist in maintaining good circulation (Chevallier, 1996).

Flavonoids have attracted interest as possible antioxidants both for inhibiting oxidative deterioration of food stuffs (Rauha *et al.*, 2000) and for providing beneficial metabolic effects in animals (Hermann, 1976). Milk thistle is rich in silydianin, silychristine and silybin, three flavonoids known collectively as silymarin (Foster, 1991). Silymarin exhibits strong hepatoprotective properties and is widely used in Europe and the USA as a herbal treatment for liver diseases such as hepatitis and cirrhosis (Saller *et al.*, 2001; Wellington and Jarvis, 2001).

In utilising oxygen, unstable electrically charged molecules known as free radicals are created. Free radicals react with other molecules and promote a chain reaction of damage resulting in alteration of cell membranes and protein damage (DNA and enzymes), a process known as oxidative stress (Schonfeld *et al.*, 1997). In 1977, Bindoli *et al.* showed that silymarin strongly inhibited peroxide formation in rat liver mitochondria and microsomes, and had an activity that was 10- fold higher than vitamin E. They attributed the effect to free-radical scavenger

activity. Flora *et al.* (1998) also state that flavonoids appear to be active as free radical scavengers and stabilisers of plasma membranes.

In order to demonstrate the antioxidant properties of flavonoids in poultry, Jenkins and workers (1993) fed young chicks a diet that induced an exudative diathesis (a disorder arising form vitamin E deficiency), and tested the efficacy of various flavonoids in preventing the disorder. They found that silymarin (milk thistle) and rutin (Rutaceae family, for example lemon and bitter orange) significantly reduced ($P<0.05$) the onset of exudative diathesis in chicks.

Tannins

'Tannin' is a generic description for a group of phenolic substances capable of tanning leather (Cowan, 1999). Tannins are ubiquitous in the plant kingdom (Chevallier, 1996) and play a prominent role in the general defence strategy of plants (Macrae *et al.*, 1993). They cause astringency, which involves contraction of body tissues and thus improves their resistance to infection (Hoffmann, 1988). Tannins are usually known mainly for their antinutritional factors, but they are thought to improve monogastric performance at low inclusion rates (McCartney, 2002).

Tannins are reported to increase feed palatability, protect mucous membranes and reduce the incidence of diarrhoea in pigs (Best, 2000). This is partially corroborated by Offiah and Chikwendu (1999) who demonstrated that extracts of *Ocimum gratissimum* (a tannin-rich Nigerian shrub of the Labiateae family) leaves inhibited castor-oil induced diarrhoea in rats, an effect thought to be elicited by the inhibition of intestinal motility. Although speculative,

tannins are considered partially responsible for the antibacterial activity of *Terminalia alata*, a traditional Nepalese medicinal plant (Taylor *et al.*, 1996).

2.3.4 Other Compounds

Mucilage

Mucilage is made up of polysaccharides that absorb water to produce a viscous mass (Bruneton, 1995; Chevallier, 1996). Mucilage lines the mucous membranes of the digestive tract, lungs and kidneys protecting against irritation, acidity and inflammation (Macrae *et al.*, 1993; Gill, 1999). McCartney (2002) states that plants rich in mucilage, such as fenugreek seeds (*Trigonella foenum graecum*) have bacteria binding properties which also aid mucosa protection. Fenugreek seeds provide gastric protection against ethanol, a necrotising agent, in rats (Pandian *et al.*, 2002). However, this effect may be at least partially attributed to the flavonoid-derived antioxidant activity of fenugreek seeds which contain high levels (>100mg $100g^{-1}$ seeds) of flavonoids (Nair *et al.*, 1998). Extracts of prickly pear (*Opuntia ficus indica*), a plant purported to be high in mucilage, have a protective effect against ethanol-induced gastric ulcers in rats (Galati *et al.*, 2001).

Glucosinolates

Glucosinolates are sulphur containing compounds commonly found in the Cruciferae family (specifically within the genus *Brassica*) (Macrae *et al.*, 1993). Glucosinolate content varies according to stage of development, but is usually highest during the period of most active growth (Fahey *et al.*, 2001). The primary biological function of glucosinolates is in plant defence against fungal and bacterial attack (Macrae *et al.*, 1993).

'Bitter' Substances

'Bitters' comprise a varied group of constituents, including sesquiterpenes and saponins (Macrae *et al.*, 1993; Harborne *et al*, 1999; Thomas, 2000), linked only by their bitter taste (Bruneton, 1995). Anecdotal evidence indicates that the bitterness stimulates secretions by the salivary glands and digestive organs, which can dramatically improve appetite and strengthen the overall function of the digestive system (Chandler, 1989; Chevallier, 1996; Hoffmann, 1998; Gill, 1999; McCartney, 2002). Bitters are present in yarrow (*Achillea millefolium*), which also possesses anti-inflammatory properties (Chandler, 1989; Bruneton, 1995; Chevallier, 1996). Cross *et al.* (2002) reported that feeding yarrow herb to growing broilers (10g kg^{-1}) significantly increased feed intake ($P<0.05$), weight gain ($P<0.001$) and FCE ($P<0.05$). However, these improvements were not observed when yarrow was fed as an essential oil (same experiment).

'Pungent' Substances

'Pungent' substances contain a wide range of active constituents grouped together by their pungent taste (Macrae *et al.*, 1993; Harborne *et al.*, 1999; Thomas, 2000). Noted examples include garlic (*Allium sativum*), the *capsicum* family (including red and chile peppers) and black pepper (*Piper nigrum*) (Bruneton, 1995). Pungent substances are reported to stimulate blood circulation, enhance immune response and have strong antibacterial properties (McCartney, 2002). *Capsicum* species are reported to stimulate saliva flow and overcome loss of appetite, stimulate gastric juice production and increase peristalsis (Macrae *et al.*, 1993), and possess a wide spectrum of antimicrobial activity (Cichewicz and Thorpe, 1996). The substance responsible for pungency in members of the *Capsicum* family is capsaicin (Bosland and Vatava, 2000). Incorporation of capsaicin at levels up to 20mg kg^{-1} in practical broiler rations does not have any deleterious effects on meat flavour (Sams *et al.*, 1995).

Ilsley *et al.* (2002) investigated the effect of adding a combination of plant extracts (1% capsicum, 1.25% cinnamaldehyde, 0.85% oregano oil) to sow diets during lactation (10g kg^{-1}). No significant effects on sow feed intake or back fat losses were observed during lactation (0-21 days), but piglet weight gain improved (days 0-1, $P<0.01$; days 1-14, $P>0.05$; days 15-21, $P<0.001$) compared with controls, indicating a greater efficiency in the use of feed for milk production.

Jamroz *et al.* (2003) studied a similar combination of plant extracts (1.98% capsaicin, 4.95% carvacrol, 2.97% cinnamaldehyde) in growing broiler chickens. This combination of plant extracts was postulated to have a positive effect on appetite and digestion, and reduce pathogenic bacteria. At 21 days of age chicks fed plant extracts at 150 and 300mg kg^{-1} were 5.4 and 8.1% heavier respectively in comparison with unsupplemented controls ($P<0.01$). Birds fed the plant extracts showed higher daily liveweight gain ($P<0.001$) and better feed conversion ratios ($P<0.05$) relative to control fed birds from 1 – 21 days of age. No statistically significant effects were observed on growth performance parameters from 1 – 42 days of age, but performance results for birds fed plant extracts were numerically superior compared with controls. Ileal crude fat digestibility coefficients were significantly higher ($P<0.05$) for groups fed plant extracts in comparison with controls, but there were no effects on ileal nitrogen or energy digestibility. The number of pathogenic bacteria (*E. coli* and *Clostridium perfringens*) in rectal digesta were markedly lower ($P<0.05$) in birds fed the higher level of herbal supplement in comparison with other treatments. No treatment differences in dressing weight were observed, although birds fed the higher level of plant extracts (300mg kg^{-1}) had less abdominal fat ($P<0.05$). In addition, significantly better sensory evaluation scores were obtained for meat tenderness ($P<0.001$) and juiciness ($P<0.05$) for breast and thigh meat samples taken from birds fed treatment diets containing plant extracts.

2.4 Plants Selected for Further Investigation

The current study will initially focus on six selected botanical products: garlic, horseradish, juniper, milk thistle, oregano and yarrow. These products offer a wide range of pharmacological properties, and several mechanisms of action are speculated including antibacterial action *in vivo*, liver protection and digestion stimulation.

2.4.1 Garlic (*Allium sativam* Liliaceae)

Garlic has been used for thousands of years for culinary, medicinal and spiritual purposes. It is a bulbous perennial herb, closely related botanically to the onion (*Allium cepa*). It has a tall flowering stem that reaches up to 1m in height, and has pink or purple flowers that bloom in mid to late summer (Plate 1). The bulb (Plate 1) is utilised for medicinal purposes, as it contains approximately 85% of the reported biologically active components (Lawson, 1998).

Plate 1: Garlic (*Allium sativum* Liliaceae) plant and bulb

Garlic is thought to have originated in Asia, and has been cultivated in the Middle East for over 5000 years, making it one of man's first cultivated plants. It is now grown successfully all around the world. The bulbs are harvested in the autumn when the flowers die and the stalk begins to wither. Garlic has many pharmacological effects, including hepatoprotective, anti-hypoglycaemic, antimicrobial and antioxidant properties (Hahn, 1996; Blumenthal, 1998). It is reported to be beneficial in the treatment of gastrointestinal and cardiovascular disorders (Blumenthal, 1998; Leporatti and Ivancheva, 2003), and has been used for thousands of years to treat a wide range of malaises, including diarrhoea, dysentery, tuberculosis, coughs, colds, bronchitis, high blood pressure and fevers (Chevallier, 1996; Leung and Foster, 1996). The German E monograph recommends daily doses of 6-10mg of alliin, which can be found in one clove of fresh garlic, or in 500-1000mg of garlic powder (Blumenthal, 1998).

Plant components and their properties

Garlic contains at least 33 sulphur compounds, several enzymes, 17 amino acids, and minerals (Block, 1985; Block, 1992; Sendl, 1995). It contains a higher concentration of sulphur compounds than any other of the *Allium* species. The sulphur components are responsible for both garlic's pungent odour and many of its medicinal effects. The composition of garlic is highly variable, depending on soil, location, season and preparation prior to analysis, among other things (Block, 1985; Sendl, 1995; Lawson, 1996; Ross *et al.*, 2001). The main components present in garlic can be segregated into sulphur and non-sulphur components (Table 2.3).

Table 2.3 Compounds found in garlic (*Allium sativum*)

Sulphur compounds:	Non-sulphur compounds:
Alliin and other cysteine sulphoxides	Alliinase and other enzymes
Glutamylpeptides	Amino acids and proteins
Allicin and other thiosulphinates	Lipids
Ajoenes	Steroids
Sulphides	Vitamins
Vinyldithins	Minerals and trace elements

Sendl, 1995; Lawson, 1996; Singh *et al.*, 1998; Holub *et al.*, 2002

One of the biologically active components, allicin (diallyl thiosulphinate or diallyl disulphide) does not exist in garlic until it is crushed or cut (table 2.4); injury of the bulb activates the enzyme alliinase which metabolises alliin to allicin (Block, 1985). Alliin is not biologically active (Stoll and Seebeck, 1948). However, allicin, which was first chemically isolated in the 1940s and is widely considered to be the most important biologically active compound in garlic (Sendl, 1995; Lawson, 1996), is effective against many bacteria, viruses, fungi and parasites (Block, 1985; Feldberg *et al.*, 1988; Sendl, 1995; Lawson, 1998; Ross *et al.*, 2001). Allicin is further metabolised into vinyldithiines, which do not appear to be biologically active (Sendl, 1995). This breakdown occurs within hours at room temperature and within minutes during cooking (Blania and Spangenberg, 1991). Indeed, Shashikanth *et al.* (1986) identified that feeding raw garlic to rats resulted in significantly lower ($P<0.05$) log counts for caecal *Streptococci*, coliforms and total bacteria in comparison to rats fed both unsupplemented control diets and diets containing boiled garlic, indicating that the antimicrobial activity of garlic *in vivo* is reduced following cooking.

Table 2.4 Alliin and allicin in whole and crushed raw garlic (mg g^{-1} fresh weight)

	Whole	Crushed
Alliin	6-14	nd
Allicin	nd	2.5-4.5

Lawson, 1996; nd=not detected

In rats, dietary alliin is rapidly absorbed and maximum serum concentrations are reached within ten minutes of administration, and it is completely excreted within about six hours. Allicin and vinyldithiines are absorbed more slowly, reaching peak serum levels between 30 and 120 minutes and persisting in the body for up to 29 days (Lachmann *et al.*, 1994).

Studies conducted on the comparative action of raw garlic extract and tetracycline hydrochloride showed raw garlic extract to be a more potent antimicrobial agent against the faecal microflora of rats than tetracycline (Shashikanth *et al.*, 1984). Further studies by Shashikanth *et al.* (1985) testing the effect of garlic, specifically allicin, on rat intestinal and caeecal microflora populations, revealed that aerobes were more susceptible to allicin concentration in the gut than anaerobes. Sharma *et al.* (1977) noted a marked reduction in the viable count of gram negative faecal bacteria within 24 hours in chickens given oral garlic supplements.

Use in Animal Production

Garlic possesses a wide range of pharmacological properties, and several controlled experiments have been conducted to examine the efficacy of garlic as a growth promoter in monogastric nutrition. The literature indicates highly variable responses to dietary garlic, with

either a positive growth response (Qureshi *et al.*, 1983a; Horton *et al*, 1991b; Mottaghitalab, 2000; Tucker, 2002; Demir *et al.*, 2003) or no growth response (de Frietas *et al.*, 2001; Al-Homidan, 2004; Cross *et al.*, 2004b) noted. No reference to garlic having a negative effect on growth performance could be found in the literature. Despite several preliminary investigations of garlic as a growth promoter, the exact modes of action are not clear.

Al-Homidan (2004) studied the effects of feeding diets containing two inclusion levels of garlic, onion or ginger (20g kg^{-1} and 60g kg^{-1}) on the growth performance and pathophysiological and blood parameters of broiler chickens between 1 and 7 weeks of age. No growth promoting effects were noted for any of the spice additives when compared to untreated controls. After 6 weeks of supplementation, ascites, mild catarrhal enteritis, individual cell necrosis of hepatocytes and degeneration of the renal tubular cells was observed in broilers fed dietary treatments containing onion and ginger, but not in chickens fed garlic or unsupplemented control diets.

Cross *et al.* (2004a) studied the effect of 7 herbal products, including garlic (10g kg^{-1}) on nutrient digestibility in 21 day old broilers. Feeding garlic supplemented diets did not result in any significant changes in the digestibility of dry matter, nitrogen, energy or organic matter relative to controls, although birds fed garlic supplemented diets had the highest digestibility values for all measured parameters during the experiment ($P>0.05$). Growth performance data taken from the same birds (Cross *et al.*, 2004b) revealed that garlic supplementation resulted in the highest body weight gains in the experiment at 7 days of age ($P<0.001$) and numerically highest ($P>0.05$) at 21 days of age. Similar findings were reported by Catala *et al.* (2004), who studied the effect of plant extracts on intestinal morphology and apparent digestibility of dry matter and crude protein in 42 day old broilers. One of the plant extracts evaluated was

capsaicin, from paprika, which also has pungent properties (McCartney, 2002). They found that feeding diets containing capsaicin increased dry matter digestibility ($P<0.001$) and crude protein digestibility ($P<0.001$) by 12.8 and 9.9% respectively relative to birds fed unsupplemented control diets. It was postulated that this increase in digestibility was mediated through increased assimilation of nutrients, as villus surface area was increased by nearly 18% ($P<0.001$) in birds fed capsaicin relative to those fed unsupplemented diets.

Demir *et al.* (2003) fed a commercial preparation containing garlic powder (Nor-Spice ® S Garlic Powder; 1g kg^{-1}) to broilers from day old to 6 weeks of age, using a commercial AGP (1g kg^{-1}) for comparison. The growth performance of birds fed the garlic supplemented diets was superior throughout the experimental period, although not statistically proven ($P>0.05$). However, ileal crypt depth in 42 day old birds fed dietary garlic was approximately 13% less than those fed the AGP ($P<0.05$), indicating a reduction in the turnover of epithelial tissue following garlic supplementation. Populations of caecal *E. coli* were not affected by dietary treatment ($P>0.05$), probably as a result of the large variation in caecal *E. coli* counts seen between birds.

Grela *et al.* (1998) fed a blend of three herbs, including garlic, to pigs during the growing (25-65kg body weight) and finishing (65-105kg body weight) period at the rate of 50g kg^{-1}. They found that feeding the supplement improved average daily gains ($P<0.05$) in the growing and finishing periods by 5 and 6% respectively, in comparison with unsupplemented groups, with an approximate 10% improvement in FCR ($P<0.05$). Qureshi and workers performed a series of experiments in chickens (1983a; 1983b) and pigs (1987) examining the effect of dietary garlic supplementation on growth performance and cholesterol metabolism. In pigs, garlic supplementation over a 4 week period (3.15g kg^{-1}; gilts 6-10 weeks of age) did not affect

measured growth parameters relative to controls, but did result in a reduction in the level of serum cholesterol ($P<0.05$). Similar effects were observed in layers (Qureshi *et al.*, 1983b) and broilers (Qureshi *et al.*, 1983a; Qureshi *et al.*, 1983b). Yalcin *et al.* (2004) studied the effects of dietary garlic (5 and 10g kg^{-1}) in laying hens and quails. They found that garlic supplementation increased egg weight ($P<0.01$) and reduced yolk cholesterol ($P<0.01$) in both species.

Proposed Mechanisms of Action

The beneficial effects of including garlic in the diet may indicate an antimicrobial effect *in vivo*, as observed under simulated conditions (Ross *et al.*, 2001). The antimicrobial property of garlic *in vivo* has been demonstrated in chicks (Haenel *et al.*, 1962) and rats (Shashikanth *et al.*, 1986), which likely explains its widespread popularity in human herbal medicine for use in combating pathogenic organisms (Chevallier, 1996; Blumenthal, 1998; Leporatti and Ivancheva, 2003). Published studies have ascribed the antimicrobial mechanism of garlic to an inhibition of sulphur dependent enzymes (Wills, 1956; Barone and Tansey, 1977; Ankri and Mirelman, 1999). Earlier work by Small *et al.* (1947) revealed that the thio-sulphide link is essential for bacterial cell function. More recent work carried out by Feldberg *et al.* (1988) has indicated that although allicin generally inhibits sulphur dependent enzymes, its specific action is concerned with inhibition of RNA synthesis in bacterial cells. This is in agreement with the views of Ankri and Mirelman (1999), who concluded that the wide-spectrum antimicrobial effects of allicin are due to the inhibitory effects on sulphur dependent enzyme systems and its interference with DNA transcription and protein synthesis.

According to a review by Hahn (1996), garlic stimulates hydrochloric acid and pancreatic enzyme production. This may explain the improvements in nutrient digestibility reported by several authors when garlic and other pungent plant extracts are fed to broilers (Catala *et al.*, 2004; Cross *et al.*, 2004a). Catala *et al.* (2004) suggested that improvements in digestibility may also be mediated by an increase in villus surface area thus improving nutrient assimilation and subsequent digestibility values. However, this is in contrast to the findings of Demir *et al.*, (2003), who demonstrated that dietary garlic resulted in reduced ileal crypt depths. His team postulated that the change in crypt depth reduces the amount of energy needed for gut maintenance, and thus increases the amount available for growth.

Despite extensive investigation into garlic as a growth promoter, little has been discovered about the likely mode of action, although it seems plausible that the primary mechanism involves antibacterial action. Garlic grows in many parts of the world and has been employed as a food seasoning in many countries. Its non-toxic nature at usual concentrations combined with its broad-spectrum antimicrobial quality present it as a plant extract worthy of further investigation.

2.4.2 Horseradish (*Armoracia rusticana* Brassicaceae)

Horseradish is a hardy perennial growing to about 50cm in height (Chevallier, 1996) (Plate 2) which is native to Europe and western Asia (Leung and Foster, 1996). The herb is widely cultivated for its rhizome, which is unearthed in the autumn. The rhizome is well known as a versatile condiment with its hot and pungent flavour, and is also popular as a medicinal herb.

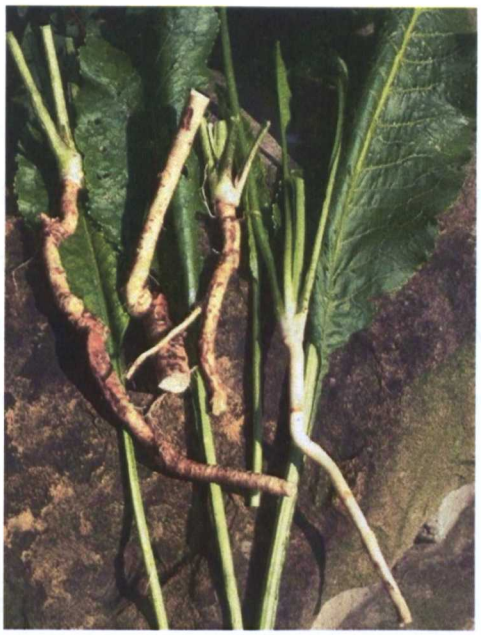

Plate 2 *Armoracia rusticana* (Horseradish)

Horseradish has GRAS certification, and in Germany it is approved in the Commission E monographs for the treatment of infections of the respiratory tract and as a supportive treatment in urinary tract infections (Blumenthal, 1998). It has also been used in North American Indian medicine; the Cherokee people use it as a respiratory aid to treat asthma, and as a gastrointestinal aid to improve digestion (Moerman, 1998).

Horseradish is mainly known for its anti-inflammatory and antimicrobial properties (Loewenfield and Black, 1978; Chevallier, 1996; Depree *et al.*, 1997), and its distillates have been shown to be effective against various bacteria and fungi, including *Listeria monocytogenes* and *Salmonella typhimurium* (Delaquis and Mazza., 1995a). The effectiveness of plants from the Brassicaceae family in the treatment of infectious diseases has been substantiated by the discovery and characterisation of antimicrobial compounds from these plants (Delaquis and Mazza, 1995b; Shofran *et al.*, 1998). The herb has been used historically

as a treatment for the common cold, hay fever, and for respiratory and urinary infections (Loewenfield and Black, 1978; Chevallier, 1996; Depree *et al.*, 1997). Horseradish is also reported to stimulate appetite and digestive functions (Loewenfield and Black, 1978; Chevallier, 1996; Moerman, 1998; Leporatti and Ivancheva, 2003). More recently, attention has focussed on the Brassica vegetables as high consumption levels have been associated with decreased levels of colo-rectal cancer (Nastruzzi *et al.*, 1996).

Plant Components and Properties

The principal components responsible for the antimicrobial action exhibited by horseradish are glucosinolate derivatives. Sinigrin is the major glucosinolate compound present in horseradish (Chevallier, 1996). When crushed or injured, the glucosinolates present in Brassicaceae plants are hydrolysed by a group of enzymes referred to as myrosinases (Delaquis and Mazza, 1998). For example sinigrin is rapidly degraded by myrosinase to yield allyl isothiocyanate (the principle degradation product), allyl thiocyanate and allyl cyanide (Tsao *et al.*, 2000). Typically, 100 grams of wet rhizome yields approximately 130mg allyl isothiocyanate (Yu *et al.*, 2001), which has been shown to exert antimicrobial effects on a wide range of gram positive and negative bacteria (Brabban and Edwards, 1995; Shofran *et al.*, 1998). Levels of allyl isothiocyanate are said to vary according to environmental conditions, such as the season and age of the plant (Mellish, 1999), although no quantitative analysis of horseradish crops could be found in the literature. Brassicaceae plants have also been shown to exhibit antioxidant properties (Plumb *et al.*, 1996).

Use in Animal Production

Despite possessing properties suitable for use as a growth promoter in broiler nutrition, no reference to the use of horseradish could be found in the literature. Metabion-3®, a proprietary product (Digestic AG, Switzerland) containing 25% horseradish, is said to improve broiler growth rates, FCR and environmental conditions within broiler houses. However, the company were not able to provide growth performance data to substantiate their claims.

Horseradish is classified as a pungent herb (Chevallier, 1996), which are reported to enhance immune response, have strong antibacterial properties, stimulate pancreatic enzyme production and overcome loss of appetite (Macrae *et al.*, 1993; McCartney, 2002). Other pungent herbs include garlic and members of the *Capsicum* family, such as chile peppers (Bruneton, 1995). Both garlic and extracts of chile peppers have been reported to improve broiler growth performance (Qureshi *et al.*, 1983a; Horton *et al*, 1991b; Mottaghitalab, 2000; Tucker, 2002; Demir *et al.*, 2003; Jamroz *et al.*, 2003).

Proposed Mechanisms of Action

The proposed mechanism of action for horseradish is likely to involve the antimicrobial action of glucosinolates and their degradation products, notably sinigrin and allyl isothiocyanate. However, the exact antimicrobial mode of action for these products is yet to be elucidated. The mechanisms by which herbs exert their antimicrobial effects are variable; for instance garlic inhibits the activity of certain enzymes involved in bacteria cell viability (Wills, 1956; Barone and Tansey, 1977) and RNA synthesis within the bacteria cell (Feldberg *et al.*, 1988; Ankri and Mirelman, 1999) whereas oregano changes bacterial cell wall permeability resulting

in leakage of cellular contents and cell death (Lambert *et al.*, 2001). It is therefore difficult to speculate the plausible antibacterial mechanisms for horseradish.

Any beneficial growth performance effects observed as a result of feeding horseradish may also arise from its purported gastric stimulation properties. Increasing pancreatic enzyme secretion through dietary herbs and spices has been previously documented in rats (Platel and Srinivasan, 1996; Platel and Srinivasan, 2000) and poultry (Williams and Losa, 2001; Lee *et al.*, 2003a; Jang *et al.*, 2004). This effect may justify the extensive use of horseradish as a gastrointestinal aid in human herbal medicine.

2.4.3 Juniper (*Juniperus communis* Cupressaceae)

Juniper is a coniferous shrub growing to 1.5m (Chevallier, 1996) (Plate 3) and it is native to the temperate regions of the Northern Hemisphere (Leung and Foster, 1996). Juniper flowers from May to June, and the berries ripen in October (Bruneton, 1995). Juniper berries are widely used as spices, and the essential oil is used in perfumes, pharmaceuticals and cosmetic products, as well as a flavouring for beverages and liquors (Chatzopoulpou and Katsiotis, 1993).

Juniper is used extensively in traditional herbal medicine, with beneficial effects for the gastrointestinal system (Chatzopoulou and Katsiotis, 1993; Chevallier, 1996; Leung and Foster, 1996; Blumenthal, 1998; Foster, 2000), kidneys (Ritch-Krc *et al.*, 1996; Foster, 2000), bladder (Foster, 2000) and rheumatism (Chavallier, 1996; Leung and Foster, 1996) reported in the literature. It has been given GRAS (Generally Recognised as Safe) Status (Leung and Foster, 1996), although prolonged use can be toxic (Bruneton, 1999).

Plate 3 *Juniperus communis* (Juniper)

Plant components and their properties

Juniper essential oil is obtained by steam distillation of the crushed, dried, partially dried or fermented berries (Leung and Foster, 1996). Berries contain 0.2-3.42% essential oil (Adams, 1998), depending on growing location and month of harvest. The essential oil content of the berries is most abundant just prior to ripening (Bruneton, 1999). Over 105 constituents have been found in juniper essential oil, 77 of which have been identified (Chatzopoulou and Katsiotis, 1993). The major component is α-pinene, as seen in table 2.5. Exact compositional analysis of juniper essential oil varies according to season in which the berries are harvested more than where the berries are grown (Ochocka *et al.*, 1997; Chao *et al.*, 2000). The monoterpene components (that is sabinene, α-pinene and myrcene) appear to be the most variable (Vernin *et al.*, 1988).

Table 2.5 Variation in juniper essential oil composition (%) according to growing location and month in which the berries are harvested

Component	Ochocka *et al.* 1997			Adams, 1998	Chao *et al.*, 2000			Properties
	Poland March	Poland Oct	France Oct	Sweden	France			
					Feb	June	Oct	
α-Pinene	78.5	56.1	80.4	56.8	27.1	40.3	42.3	Antibacterial; antiinflammatory; flavour
Camphene	0.7	0.2	0.5	0.6	0.2	0.3	0.2	Antioxidant; expectorant
β-Pinene	4.4	1.6	5.3	4.4	2.2	2.8	1.7	Antiinflammatory
Sabinene	ND	6.0	ND	0.7	13.2	3.8	0.2	No activity reported
δ-3-Carene	ND	ND	ND	4.7	0.1	Tr.	ND	Antibacterial; anti-inflammatory
Myrcene	14.1	21.5	9.9	5.2	9.6	10.6	8.1	Antibacterial; antioxidant
α-Terpinene	0.1	0.5	ND	ND	0.3	0.1	ND	Antibacterial
Limonene	1.4	9.8	1.2	6.9	1.0	1.9	0.8	Bitter; flavour
γ-Terpinene	ND	0.9	ND	Tr	0.7	0.2	0.1	Antioxidant
ρ-Cymene	ND	0.6	0.1	0.3	0.3	0.1	0.1	Antibacterial

Tr.=trace; ND=not detected

Juniper berries are rich in terpenes, particularly monoterpenes (Macrae *et al.*, 1993; Tunon *et al.*, 1995; Harborne *et al.*, 1999). Chao *et al.* (2000) examined juniper berry essential oil and detected bacteriocidal properties against both gram negative (including *Enterobacter cloacae* and *Escherisha coli*) and gram positive (including *Staphylococcus aureous* and *Streptococcus faecalis*) bacteria. Juniper has also been shown to possess anti-inflammatory activity (Takacsova *et al.*, 1991; Tunon *et al.*, 1995).

Juniper berries are used by the Carrier people of British Columbia to relieve kidney infections (Ritch-Krc *et al.*, 1996). The essential oil is used as an antiseptic in both Bulgarian and Italian herbal treatments (Leporatti and Ivancheva, 2003). According to the French Pharmacopoeia,

juniper berries stimulate appetite and the production of gastric juices (Bruneton, 1999). The German Commission E Monograph recommends daily doses of 20-100mg for gastrointestinal complaints (Blumenthal, 1998).

Uses in Animal Production

No reports of feeding solely juniper as a growth promoter can be found in the literature. However, Tucker (2002) described the effects of a blend of herbal products (Apex Poultry®, Braes Feed Ingredients, UK; 150mg kg^{-1}) including juniper essential oil, on growth performance and caecal *Clostridia* counts of broilers fed wheat based diets up to 40 days of age. The supplement improved weight gains and FCR equal to that of a traditional AGP (Avilamycin) from 0-20 days of age, with both additives exceeding ($P<0.05$) the performance levels attained by birds fed unsupplemented control diets. In addition, feeding the herbal supplement reduced caecal *Clostridia* counts at 40 days of age relative to unsupplemented controls. However, Apex Poultry® is a blend of six herbal products, and the effects of juniper essential oil in isolation were not examined. Deyoe *et al.* (1962) gave growing broilers access to both plain water and water containing one of eleven flavours (including juniper) and assessed the effects on bird growth, feed intake and FCR. They found that the juniper-flavoured water was the only flavoured water to be consumed in excess of plain water, and that it increased 4 week broiler weight and improved FCR.

Although there are no broiler growth studies using juniper exclusively, it is used widely in human herbal medicine, both as an antiseptic and for gastric disorders. In addition, there are anecdotal claims (McCartney, 2002) that juniper essential oil possesses appetising and digestion enhancing properties in broilers. Essential oils have been extensively examined as

alternative growth enhancers, with various growth performance effects observed. Unfortunately, a large amount of the experimental research carried out on the effects of feeding essential oils has been proprietary in origin, and as such the exact composition of the essential oil blends fed are subject to secrecy. The majority of essential oil blends appear to contain either oregano or thyme, or both, and are typically included at levels of between 50 and 500mg kg^{-1}. Numerous studies have highlighted the growth performance benefits of feeding essential oils (Piva *et al.*, 1991; Williams and Losa, 2001; Tucker, 2002; Alcicek *et al.*, 2003; Jamroz *et al.*, 2003; Jang *et al.*, 2004) with others describing no positive effects (Piva *et al.*, 1991; Jamroz *et al.*, 2003; Lee *et al.*, 2003a; Hernandez *et al.*, 2004). No reports of negative growth performance effects following dietary supplementation of essential oils were found in the literature.

Proposed mechanisms of action

Juniper posseses strong antimicrobial properties *in vitro*, so it is likely that any positive effects as a result of feeding it to broilers would be mediated through modulation of the gut microflora. This is consistent with the proposed mode of action for oregano (Sivropoulou *et al.*, 1996; Lambert *et al.*, 2001), which has been extensively researched as a herbal growth promoter in monogastrics. In addition, there is evidence that essential oils have digestion enhancing properties (McCartney, 2002). A variety of positive physiological responses to dietary supplementation of various essential oil blends have been described, including increased digestive enzyme activity (Lee *et al.*, 2003a; Jang *et al.*, 2004), increased nutrient digestibility (Jamroz *et al.*, 2003), reductions of pathological gut microflora, such as *Clostridium perfringens* and *E. coli* (Jamroz *et al.*, 2003) and reduced abdominal fat retention

(Jamroz *et al.*, 2003). In addition, positive dose responses with inclusion rates of up to 300mg kg^{-1} feed have been noted (Alcicek *et al.*, 2003; Jamroz *et al.*, 2003).

2.4.4 Milk Thistle (*Silybum marianum* Gaertner)

Milk thistle (*Silybum marianum* (L.) *Gaertner*) is a member of the Aster family (Asteraceae or Compositae) (Thomas, 2000). Synonyms for the plant include Mary's Thistle, Saint Mary's Thistle, Marian Thistle, Lady's Thistle and Holy Thistle (Foster, 1991). Milk thistle is a spiny biennial, growing up to 1.5m in height, with white veined leaves and purple flowerheads (Chevallier, 1996) (Plate 4). Mature flowerheads can reach 6cm in diameter, and contain shiny black seeds which are used in herbal preparations (Leung and Foster, 1996). Milk thistle is a widespread wayside herb of uncultivated ground and waste places throughout much of Europe, and is naturalised in the Eastern United States, California and South America (Foster, 1991; Flora *et al.*, 1998). It is grown commercially throughout the United States, preferring sunny locations and well drained soil (Flora *et al.*, 1998).

Plate 4 Milk Thistle (*Silybum marianum*)

Milk thistle has been cultivated in European gardens as a vegetable, where the young leaves are used in salads and as a spinach substitute. Young stalks are peeled and soaked and eaten like asparagus, roots are soaked overnight and eaten, flower heads are eaten like artichokes, and the roasted seeds have been used as a coffee substitute (Foster, 1991). The plant has been used medically for more than 2000 years, and it is still used today. It is classified as "generally recognised as safe" (GRAS). The German Commission E monograph on milk thistle seed recommends preparations of the seeds for the supportive treatment of chronic inflammatory liver disorders and cirrhosis of the liver, and for dyspeptic complaints (Blumenthal, 1998). Milk thistle is also used as a supportive treatment of chronic liver disorders such as cirrhosis, hepatitis and damage as a result of alcohol and toxic chemicals (Fintelmann, 1991; Flora *et al.*, 1998; Kvasnicka *et al.*, 2003). This is due to its protective effects on the liver when exposed to various toxins such as phalloidin, galactosamine, halothane and carbon tetrachloride. It has also been shown be beneficial in the treatment of gastrointestinal disturbances, and is reported to have positive effects on the immune system (Foster, 1991; Valenzuela and Garrido, 1994; Blumenthal, 1998; Saller *et al.*, 2001), as well as offering protection from asthmatic disorders (Breschi *et al.*, 2002). Traditionally, milk thistle has been used for its hepatoprotective properties and for the treatment of urinary tract and bladder diseases in Bulgarian and Italian herbal medicine (Leporatti and Ivancheva, 2003).

Plant components and their properties

There are in excess of 500 known flavonoids in the plant and animal kingdom (Valenzuela and Garrido, 1994), and they are used extensively in herbal medicine (Bito *et al.*, 2002) for their hepatoprotective (Rui, 1991; Saller *et al.*, 2001), anti-oxidant (Valenzuela and Guerra, 1986; Nieto *et al.*, 1993), anti-inflammatory (Gupta *et al.*, 1999; Wellington and Jarvis, 2001) and

anti-dyspeptic (Fintelmann, 1991) properties. Wagner and workers (1968) were the first to isolate a mixture of active principles from milk thistle seeds, which they characterised as silymarin (Tyler, 1998). Silymarin is composed mainly of three flavonoid isomers, namely silybin (also known as silibinin, silybinin or silibin), silydianin (silidianin) and silychristin (silichristin) (figure 2.2). Other flavonoligans present in the seeds include dehydrosilybin, desoxysilichristin, desoxysilydianin, silandrin, silybinome, silyhermin and neosilyhermin (Kvasnicka *et al.*, 2003). However, it is widely accepted that silybinin has the most biological activity (Valenzeula and Garrida, 1994, Rui, 1991; Flora *et al.*, 1998). Milk thistle also has 'bitter' properties (Leung and Foster, 1996), but the compound responsible for this bitter action has not been identified.

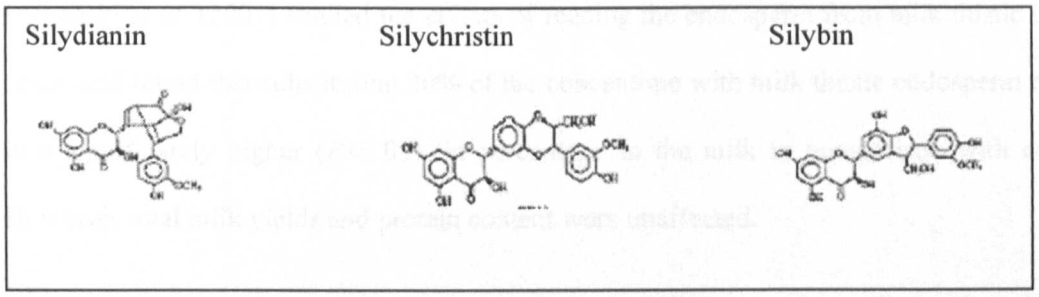

Figure 2.2. Structures of the three flavonoid isomers collectively known as silymarin

Use in animal production

Only one reference to the use of milk thistle as a monogastric herbal growth promoter could be found in the literature. Dobretsberger *et al.* (1997) compared Livol© (Indian Herbs, Saharanpur), a proprietary product containing milk thistle, with a traditional AGP (tetracycline) in an experiment conducted under practical conditions on a farm. Approximately 37,000 birds (0–42 days of age) were involved in the experiment, but unfortunately, no

negative control was used. They reported that feed consumption and body weight gain were similar for the two groups resulting in an overall FCR of 1.87 and 1.85 ($P>0.05$) for AGP and herb supplemented groups respectively. After slaughtering 10 random birds from each group, analysis revealed that birds fed the herbal supplement achieved higher carcass weights than birds fed the AGP supplemented diets (1240 and 1101g carcass weight for herb supplemented and control fed birds respectively, $P<0.05$). However, the absence of a negative control in this study may negate the results as it does not prove that the growth performance of birds fed either of the diets was superior to what it would be in birds fed unsupplemented diets. Nevertheless, due to the lack of experimental work performed in this area, it is worth considering their findings.

Potkanski *et al.* (2001) studied the effects of feeding the endosperm from milk thistle to dairy cows, and found that substituting 20% of the concentrate with milk thistle endosperm resulted in a significantly higher ($P<0.05$) fat percentage in the milk in comparison with controls. However, total milk yields and protein content were unaffected.

In order to demonstrate the antioxidant properties of flavonoids in poultry, Jenkins and workers (1993) fed young chicks a nutritional muscular dystrophy (NMD; a disorder arising from vitamin E deficiency) inducing diet, and tested the efficacy of various flavonoids in preventing the disorder. They found that silymarin reduced ($P<0.05$) the onset of NMD in chicks.

Additionally, silymarin supplementation has been shown to affect bile salt secretion, which may be as a result of its bitter qualities (Leung and Foster, 1996). Bile salts play a crucial role both in bile formation and, once delivered to the intestine, in lipid absorption by the intestinal

tract (Leeson, 2001). Crocenzi *et al.* (2000) demonstrated that dietary silymarin induced a dose dependent increase in bile salt secretion in Wistar rats (+49% in comparison with controls, $P<0.05$), with the maximal effect being reached at a dose of 100mg kg day^{-1}. They suggested that silymarin increases the bilary secretion of the endogenous pool of bile salts by stimulating the synthesis of hepatoprotective bile salts, such as β-muricholate and urseodeoxycholate. Khan *et al.* (1986) studied the effect of feeding milk thistle seed oil (5 and 10mg kg^{-1}) on growth and microscopic lesions in mice. They found that dietary inclusion of milk thistle resulted in higher body weight gains during the 6 week experimental period and increased feed intake. No additive effects on growth performance were observed. *Post mortem* examination of the mice at the end of the experiment did not find any microscopic pathological lesions (liver, kidney, stomach and intestines), indicating no toxicity at the levels fed.

Proposed mechanisms of action

The primary use of milk thistle seems to be as a hepatoprotectant. A review of available literature seems to suggest three main mechanisms of action:

Antioxidant activity

Silymarin exhibits strong antioxidant activity, and possesses a free-radical scavenging activity 10-fold greater than vitamin E (Bindoli *et al.*, 1977). This scavenging ability can most likely be attributed to the presence of hydroxyl groups in different positions in their benzene rings which enables them to neutralise free radicals (Nieto *et al.*, 1993).

Regulation of cell membranes

Studies on the effect of silymarin on the liver have demonstrated that it acts on liver cell membranes to regulate the cellular membrane permeability. This increases the cell's

stability, and prevents the entry of virus toxins and other toxic compounds and thus prevents damage to the cells (Foster, 1991; Leung and Foster, 1996; Grange *et al.*, 1999).

Enhanced protein synthesis

Silymarin improves liver regeneration when the body is affected by liver diseases, such as hepatitis, cirrhosis and mushroom poisoning (Letteron *et al.*, 1990; Valenzuela and Garrido, 1994). This is probably due to its stimulatory action on the activity of nuclear polymerase A, which results in increased ribosomal protein synthesis, and thus stimulates the regeneration activity of the liver and the formation of new hepatocytes (Foster, 1991; Blumenthal, 1998; Horvath *et al.*, 2001; Wilasrusmee *et al.*, 2002).

Milk thistle is of interest in this study because it has strong hepatoprotective properties, and may have a positive effect on fat digestibility *in vivo* as a result of its ability to increase bile salt secretion. In addition, there is no documentation of the herb having any toxic effects. Although there are no reports of controlled experiments involving milk thistle dietary supplementation of broiler chickens in the literature, the field experiment conducted by Dobretsberger *et al.* (1997) described positive growth performance results, and milk thistle has been shown to have a positive effect on growth performance in mice (Khan *et al.*, 1996).

2.4.5 Oregano (*Origanum vulgare* Labiatae)

The *Origanum* (*Labiatae* family) genus consists of 38 species widespread in the Mediterranean region (Aligannis *et al.*, 2001). Eleven species occur in Greece, five of which are found in Crete (Skoula *et al.*, 1999). Members of the *Origanum* species are characterised by a wide range of volatile secondary metabolites and by the existence of wide differences, both with respect to essential oil content and composition (Thomas, 2000). As a result of these

discrepancies, only one *Origanum* species will be reviewed and investigated: *Origanum vulgare* subsp. Hirtum (oregano). Oregano, also known as Wild Marjoram, is an upright perennial herb growing to about 80cm (Thomas, 2000) (Plate 5). It is native to Europe, and naturalised in the Middle East, thriving on chalky soils in coastal areas (Chevallier, 1996).

According to Blumenthal (1998) oregano is effective against gastrointestinal disturbances and bloating, and stimulates appetite and digestion. The herb is used extensively in Bulgarian and Italian phytomedicine to aid effective digestion and promote gastric secretion (Leporatti and Ivancheva, 2003).

Plate 5 *Origanum vulgare* subsp. Hirtum (Oregano)

Plant Components and Their Properties

Several recent studies have shown that aromatic herb plants, particularly those of the *Labiatae* family possess varying antioxidative, antimicrobial and antifungal activity (Economou *et al.*, 1991; Sivropoulou *et al.*, 1996; Adam *et al.*, 1998). Oregano is of particular interest as its dry

leaves and flowers, its extracts with organic solvents, and the essential oil obtained by steam distillation have all been reported to possess substantial antimicrobial, antifungal and antioxidative activity (Tsimidou *et al.*, 1995; Botsoglou *et al.*, 2002).

Oregano contains 0.1-1.0% essential oil (Thomas, 2000), the major components being the monoterpene lactones carvacrol (5-isopropyl-2-methylphenol) and thymol (5-methyl-2-isopropylphenol) (Figure 2.3) (Adam *et al.,* 1998) with highly variable relative proportions (Table 2.6).

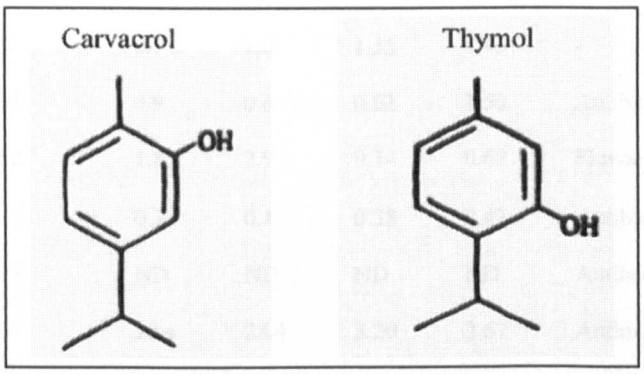

Figure 2.3 Structure of thymol and carvacrol

Oregano essential oil is usually obtained through steam-distillation, and yields more than 30 components, many of which are phenolic antioxidants (Vekiari *et al.*, 1993). The two major components carvacrol and thymol typically constitute about 78-82% of the total oil (Adam *et al.*, 1998), but this is highly variable (table 2.6). Variation in composition of the oil arises from differences in genotype (Piccaglia *et al.*, 1993), climate (Boira and Blanquer, 1998), seasonality (Kokkini *et al.*, 1997), environmental factors such as cultivation, temperature and photo-period (Tucker and Maciarello, 1994) and storage conditions (Bruneton, 1999). Both

carvacrol and thymol exhibit considerable antimicrobial and antifungal activity (Deans and Svoboda, 1990; Sivropoulou *et al.*, 1996; Dorman and Deans, 2000).

Table 2.6 Variability in oregano essential oil composition

Component	Composition (%)					Pharmacological effect
	Sivropoulou *et al.*, 1996	Baratta *et al.*, 1998	Dorman *et al.*, 2001	Skoula *et al.*, 1999 Sample 1	Skoula *et al.*, 1999 Sample 2	
α-pinene	0.88	0.7	Tr.	0.69	0.77	Antibacterial; immunostimulant
Camphene	0.15	0.2	ND	0.40	0.22	Antioxidant; expectorant
β–pinene	0.08	2.0	Tr.	0.30	0.34	Anti-inflammatory; antiseptic
Sabinene	0.04	0.1	1.0	1.35	1.45	-
Myrcene	0.61	0.9	0.6	0.62	1.50	Antibacterial; antioxidant
α-terpinene	0.62	1.8	2.9	0.34	0.68	Flavour
Limonene	0.14	0.3	0.4	0.38	0.42	Antibacterial; antiseptic
1,8-cineole	0.18	ND	ND	ND	ND	Antibacterial; hepatotonic
γ–terpinene	2.07	10.4	28.4	3.20	2.67	Antioxidant
p-cymene	8.76	11.5	2.1	14.70	11.91	Antibacterial; antiviral
Linalool	0.12	0.1	4.6	0.52	0.40	Antibacterial; antiseptic
β–caryophyllene	1.50	0.4	1.2	0.81	0.24	Antibacterial; anti-inflammatory
α-terpineol	0.42	0.3	4.6	ND	0.11	Antibacterial
γ–cadinene	0.02	ND	ND	ND	ND	Flavour
Thymol	2.45	32.4	19.7	0.62	0.28	Antibacterial; antioxidant
Carvacrol	79.58	21.5	8.2	53.19	72.04	Antibacterial; antioxidant

ND = Not Detected; Tr. = trace

The activity of other constituents, such as the two monoterpene hydrocarbons γ-terpinene and ρ-cymene, which account for approximately 5% and 7% of the essential oil respectively

(Adam *et al.*, 1998) is uncertain, as is the possible synergistic effect of all the constituents put together. A high amount of carvacrol (79.58%) is frequently observed in several Greek wild populations (Sivropoulou *et al.*, 1996), but in some cases thymol, instead of carvacrol, appears as the major component of the Greek oregano essential oil (Adam *et al.*, 1998; Baratta *et al.*, 1998).

Numerous studies have confirmed that carvacrol and thymol are bactericidal to pathogenic micro-organisms and in particular to *E. coli* (Deans and Svoboda, 1990; Sivropoulou *et al.*, 1996; Helander *et al.*, 1998; Ultee *et al.*, 1998; Hammer *et al.*, 1999; Dorman and Deans, 2000; Skandamis *et al.*, 2002). The routine test used to assess the effectiveness of antibacterial agents is to determine the Minimum Inhibitory Concentration (MIC), which is the concentration of active substance that will enable total inhibition of bacterial growth. MIC values between 0.05 and 250μg ml^{-1} have been reported against strains of bacteria frequently found on commercial poultry farms (Sivropoulou *et al.*, 1996). Sivropoulou *et al.* (1996) demonstrated that thymol is more active than carvacrol against gram negative bacteria, such as *E. coli* and *Salmonella typhimurim.* The effect of *E. coli* in the small intestine is well documented: cell wall thickness is increased in order to reduce bacterial invasion. However a thickening of intestinal wall leads to a reduction in overall nutrient absorption and therefore in animal performance.

Use in Animal Production

To date, the proposed growth promoting effects of oregano have been well researched in pigs and poultry nutrition, but the mode of action is still not fully understood. A number of

commercial products containing Oregano are available, notably Orego-Stim® (Meriden Animal Health Limited, UK).

Waldenstedt (2000) reported that the addition of Orego-Stim® at the manufacturer's recommended inclusion rate (165mg kg^{-1}) significantly improved liveweight gain ($P<0.001$) in growing broilers from day 13 to day 48. Accumulated feed intake was also higher during this period ($P<0.005$), which the author attributes to the aromatic flavour of the oregano extract, whilst feed conversion ratio was unaffected. Demir *et al.* (2003) demonstrated that dietary supplementation with an oregano based commercial product (Nor-Spice® Oregano Powder, 100mg kg^{-1}) resulted in growth performance comparable with traditional AGP in female broiler chickens from 0-42 days of age. However, Cross and workers (2002) report that dietary oregano (100mg kg^{-1}) had no positive effect on the growth performance of growing broiler chickens between 7 and 28 days of age. This concurs with the findings of Botsgolou *et al.* (2002) and Lee *et al.* (2003a) who failed to detect a significant growth response to dietary oregano essential oil supplementation at 50 and 100mg kg^{-1} and 100mg kg^{-1} respectively. Further work by Lee *et al.* (2003b) focussed on the effects of feeding the principle active components of oregano, carvacrol and thymol, separately. Interestingly, birds fed treatment diets containing carvacrol (200mg kg^{-1}) had significantly ($P<0.05$) lower weight gains (-4.8%) and feed intakes (-7.1%) compared with birds fed thymol supplemented (200mg kg^{-1}) treatment diets, but better ($P<0.05$) feed conversion efficiency. A similar pattern was observed when carvacrol and thymol were fed to broilers infected with coccidia (*Eimeria acervulina*) (Ibrir *et al.*, 2001). However, no explanation of the variant effects of the two different isomers was offered by either group.

The influence of dietary oregano supplementation on avian coccidiosis has been examined in several studies (Ibrir *et al.*, 2001; Ibrir *et al.*, 2002; Waldenstedt, 2000; Giannenas *et al.*, 2003) with inconsistent findings. Giannenas *et al.* (2003) investigated the effect of dietary supplementation of oregano essential oil (300mg kg^{-1}) on the performance, excretion of oocysts and incidence of bloody excreta in chicks experimentally infected with *Eimeria tenella*. Prior to infection at 14 days of age, no treatment differences were observed in any of the measured parameters. However, between 14 and 42 days of age, infected birds fed oregano essential oil supplemented diets achieved significantly ($P<0.05$) higher body weights, superior feed conversion, lower incidence of bloody excreta and lower faecal oocyst output than their control fed conspecifics. Growth performance, incidence of bloody excreta and faecal oocyst output were not significantly different between uninfected birds fed control diets and infected birds fed oregano supplemented treatment diets. However, infected birds fed treatment diets containing a traditional coccidiostat (lasalocid, 75mg kg^{-1}) outperformed birds fed all other treatment diets ($P<0.05$), and had lower ($P<0.05$) incidences of bloody excreta and faecal oocyst output than other infected birds. Waldenstedt (2000) reported that caecal numbers of *Clostridium perfringens* were significantly lower (log_{10} cfu 4.1 *vs.* 6.1; $P<0.05$) in chicks fed oregano supplemented diets (330mg kg^{-1}) compared with untreated controls. However, work by Ibrir *et al.* (2001 and 2002) failed to show any effect of carvacrol (125mg kg^{-1}) or thymol (125mg kg^{-1}) supplementation on faecal oocyst output or incidence of bloody excreta in chicks experimentally infected with *Eimeria acervulina*.

Meat scientists at Aristotle University have carried out a series of experiments investigating the effects of dietary essential oil supplementation on the susceptibility of raw and cooked poultry meat to lipid oxidation (Botsgoulou *et al.*, 2002; Botsgoulou *et al.*, 2003a; Botsgoulou *et al.*, 2003b). Lipid oxidation is a major problem encountered in meat processing, particularly

with poultry meat, which is high in polyunsaturated fatty acids (Gray and Pearson, 1987). Chickens were fed diets containing 50 and 100mg kg^{-1} oregano essential oil (Botsgoulou *et al.*, 2002; Botsgoulou *et al.*, 2003a), and turkeys were fed higher levels of 100 and 200mg kg^{-1} (Botsgoulou *et al.*, 2003b). In all experiments, dietary oregano essential oil supplementation resulted in reduced ($P<0.05$) lipid oxidation of breast and thigh meat samples compared with controls. In addition, significant ($P<0.05$) dose response increases in antioxidant status of meat were found in all three experiments.

Recent studies on the use of Orego-Stim® in growing pigs have demonstrated reductions of the incidences of post weaning diarrhoea syndrome (Kyriakis *et al.*, 1998) and porcine intestinal adenomatosis (Tsinas *et al.*, 1998). This has been attributed to an alteration in gut bacterial populations, particularly E. *coli*, which is a major factor associated with the occurrence of both these ailments.

In an experiment carried out by Kyriakas *et al.* (1998) piglets were weaned at 21 days of age and fed one of three dietary treatments (control (no AGP), or Orego-Stim® at 250g $tonne^{-1}$ or 500g $tonne^{-1}$) from 8 to 21 days post weaning. Piglets fed oregano had significantly higher ($P<0.05$) daily weight gain over the experimental period (9.4% and 12.3% higher than control fed piglets for low and high inclusion rate of oregano respectively). In addition, piglets fed the higher level of oregano had higher gains than those fed the lower inclusion level (11.2% higher, $P<0.05$) indicating an additive effect of oregano supplementation. Feed conversion ratio was also improved by oregano supplementation ($P<0.05$) over the experimental period, but no difference between the oregano inclusion levels was noted.

A similar study carried out at the same institution (Tsinas *et al.*, 1998) assessed the performance of piglets fed the same three treatment diets (control, Orego-Stim® at 250g tonne^{-1} or 500g tonne^{-1}) from weaning at 21 days to slaughter at 161 days of age (table 2.7). Again, dietary oregano addition appears to be additive with respect to daily weight gain.

Table 2.7 Growth performance data of pigs following oregano supplementation (weaning to slaughter)

	Control	250g t^{-1}	500g t^{-1}
DLWG (g d^{-1})	611 ^{c}	651 ^{b}	676 ^{a}
DFI (kg d^{-1})	1.85 ^{b}	1.75 ^{ab}	1.70 ^{a}
FCR	3.03 ^{a}	2.68 ^{b}	2.50 ^{b}

Means in rows with different subscripts differ significantly ($P<0.05$); Adapted from Tsinas *et al.*, 1998

Another study (Gollnisch *et al.*, 2001) failed to demonstrate beneficial performance effects in pigs (21-56 days of age) fed treatment diets containing oregano essential oil (100mg kg^{-1}). However, a traditional AGP was used as a positive control in the same experiment, and also failed to elicit any effect on growth response. Overall pig performance was high which may have accounted for the lack of statistical significance between treatments.

Proposed mechanisms of action

Phenolic compounds, such as carvacrol and thymol, have been used as disinfectants for over one hundred years, and continue to be used today (Bruneton, 1999). Carvacrol and thymol inhibit pathogenic bacteria by damaging the protein within the cell wall of the bacteria and changing its permeability to phosphate and potassium ions (Lambert *et al.*, 2001). The dissipation of ion gradients leads to an impairment of essential processes within the bacteria cell and allows leakage of cellular constituents, which results in water imbalance and, ultimately, cell death (Heipieper *et al.*, 1991). Interestingly, Lambert *et al.* (2001) observed

cell leakage of potassium and phosphate ions where the concentration of oregano essential oil was lower than the minimum inhibitory concentration (MIC). Sivropoulou *et al.* (1996) showed that oregano essential oil halted the replication of *Staphylococcus aureus* at dilutions of 1/10000, while dilutions as high as 1/50000 still caused a considerable decrease in bacterial growth rate.

2.4.6 Yarrow (*Achillea millefolium* Linnaeus)

Achillea millefolium (Yarrow) (Plate 6) is a member of the Compositae (formerly Asteraceae) family, the largest family of flowering plants, which includes daisies, thistles and sunflowers (Mitich, 1990). It is a perennial herb, growing up to 60cm tall, and flowers from June to October (Podlech, 1996). The plate is native to Europe and Asia and is naturalised in North America (Podlech, 1996). It is typically found growing in meadows, pastures, roadsides and gardens (Leung and Foster, 1996).

Plate 6 *Achillea millefolium* - Yarrow

Yarrow has a pleasant spicy aroma and is of interest to perfumers (Loewenfeld and Back, 1978, Mitch, 1990). There are many medical applications for the herb ranging from the treatment of sprains to the treatment of the common cold. The herb is traditionally used as a digestive stimulant, to improve appetite, to settle digestion, to stimulate bile flow and liver function and for treatment of gastric and dyspeptic conditions (Loewenfeld and Black, 1978, Mills, 1993; Bruneton, 1995; Podlech, 1996; Weimann and Heinrich, 1998; Saller *et al.*, 2001; McCartney, 2002; Leporatti and Ivancheva, 2003). Yarrow is also reported to be beneficial to the kidneys (Loewenfeld and Black, 1978; Chandler *et al.*, 1982). Yarrow is utilised throughout North America by a number of Indian societies for a wide variety of medicinal purposes including as a kidney and liver aid, for treatment of indigestion and as a bitter tonic (Moerman, 1977).

Plant components and their properties

The chemical composition of yarrow has been studied extensively in an attempt to explain the biological properties of the herb, but reported compositional analysis is highly variable (Table 2.8). Yarrow has a tendency to hybridise, thus the composition varies greatly between species (Chandler *et al.*, 1982a; Guedon *et al.*, 1993; Bruneton, 1995). The composition of the essential oil also varies depending on factors such as the stage of development of the plant (Figueiredo *et al.*, 1992), and the environmental conditions (Hofmann and Fritz, 1993) and geographical location (Chevallier, 1996) in which it is grown. Yarrow contains approximately 0.1-1.4% volatile oil (Chevallier, 1996; Leung and Foster, 1996). The oil includes the terpene lactones 1,8-cineol, pinene, chamazulene, and camphene, among others (Furia and Bellanca, 1975; Chandler *et al.*, 1982a; Figueiredo *et al.*, 1992; Rohloff *et al.*, 2000). It is also reported to contain flavonoids and tannins (Guedon *et al.*, 1993; Leung and Foster, 1996).

Table 2.8 Variability in yarrow essential oil composition

Component	Composition (%)								Pharmacological effect
	Hachey *et al.*, 1990	Figuerdo *et al.*, 1992	Kokkalou *et al.*, 1992	Hofmann *et al.*, 1993	Rohloff *et al.*, 2000	Candan *et al.*, 2003	Cross, 2004 Sample 1	Cross, 2004 Sample 2	
α-pinene	ND	0.8	0.06	0.91	3.25	2.4	3.8	1.15	Antibacterial; immunostimulant; flavour
Sabinene	7.3	5.4	1.1	8.75	3.75	2.8	4.94	ND	-
β–pinene	5.8	1.1	0.18	4.99	16.2	4.2	18.54	5.06	Antibacterial; flavour; anti-inflammatory
1,8-cineole	11.45	24.5	11.87	3.45	6	24.6	ND	1.88	Antibacterial; hepatotonic
γ–terpinene	ND	1.8	0.38	2.92	1.75	1	0.50	ND	Antioxidant; flavour
Linalool	ND	ND	ND	ND	1.85	0.6	13.54	15.13	Antibacterial; flavour; antiviral
α-thujone	ND	ND	ND	10.35	10	ND	ND	ND	Antibacterial
β–thujone	15	ND	ND	4.78	1	ND	ND	ND	Antibacterial
Camphor	10.15	4.9	22.23	3.97	2.2	16.7	17.7	10.09	Antiseptic; antidiarrhoea
Borneol	ND	ND	ND	ND	1	4	2.06	4.68	Flavour; hepatoprotective; antiinflammatory
4-Terpineol	ND	5.6	ND	ND	2	2.8	0.55	1.9	Antioxidant; flavour
α-terpineol	ND	1.2	0.18	ND	2.75	10.2	3.15	ND	Antibacterial; flavour
Bornyl acetate	ND	ND	1.95	1.94	1	0.1	ND	ND	Antibacterial; flavour
Chamazulene	ND	ND	ND	17.22	ND	ND	4.8	27.94	Antioxidant; flavour; antiinflammatory
Limonene	ND	ND	ND	ND	ND	ND	3.05	4.53	Antibacterial

ND = not detected; Tr. = trace

Yarrow is used in traditional herbal medicine to prevent infections and provide local anaesthesia (Mitch, 1990). These claims are substantiated by several *in vitro* experiments which demonstrate the antibacterial action of yarrow (Bishop and MacDonald, 1951; Picman, 1986; Kedzia *et al.*, 1990; Blumenthal, 1998; Vagi *et al.*, 2002; Candan *et al.*, 2003). Kedzia *et al.* (1990) observed that yarrow exhibited antimicrobial activity against *Staphylococcus aureus* and *Candida albicans* yeasts, but not *Escherichia coli*, which is corroborated by Picman (1986). These authors attribute the antimicrobial activity to the terpene lactones, specifically the monoterpene and sesquiterpene fractions. Monoterpenes are ubiquitous in the plant kingdom, and their functions are to attract pollinators to flowers and to protect them from microbial infection (Harborne *et al.*, 1999). The monoterpenes carvacrol and thymol are largely responsible for the strong antibacterial effects of oregano observed *in vitro* (Deans and Svoboda, 1990; Sivropoulou *et al.*, 1996; Dorman and Deans, 2000). Sesquiterpenes are less common than monoterpenes, but are widespread in the Asteraceae family (Cowan, 1999). Again, these secondary metabolites play an important role in the protection of plants against pathogen attack, predation from herbivorous mammals and insects and competition with other plants (Bruneton, 1995). They show strong antioxidant (Safayhi *et al.*, 1994; Rekka *et al.*, 1996), antimicrobial and antifungal activity *in vitro* (Rodriguez *et al.*, 1976) and are stable and lipophilic compounds (Picman, 1986). In addition, they are responsible for the characteristic 'bitterness' of the yarrow plant, although the exact components responsible have not been identified (Kubelka *et al.*, 1999). Bitter herbal drugs have played an important role in the treatment of patients with dyspeptic symptoms (Blumenthal, 1998). The mechanisms of bitters are not completely understood, but there are indications that, even at very small concentrations, they stimulate the secretion of digestive enzymes (Picman, 1986; Hoffman, 1998; Saller *et al.*, 2001). Some sesquiterpenes have been reported to act as a feeding deterrent to rabbits and deer (Picman, 1986) and to be toxic in sheep (Rodriguez *et al.*, 1976).

Sesquiterpenes can constitute up to 5% of the dry weight of aerial parts of plants belonging to the Asteraceae family (Bruneton, 1995), and their bitterness has been suggested as the primary anti-feedant factor (Picman, 1986).

Use in animal production

Cross *et al.* (2002) fed yarrow supplemented (10g kg^{-1}) diets to caged male broiler chickens from 7 to 28 days of age. They reported that dietary yarrow had no effect on 7-21 day old broilers, but improved FCE ($P<0.05$) relative to unsupplemented controls between 21 and 28 days of age. The authors hypothesised that the positive growth performance responses seen were as a result of a beneficial effect on gut microflora. However, further studies by the group (Cross *et al.*, 2001) failed to demonstrate a link between yarrow supplementation and a change in *E. coli*, lactic acid bacteria or *Clostridium perfringens* populations in either faecal or caecal samples. In a more recent study (Cross *et al.*, 2004b), diets containing no herbal supplement (control) or one of seven herbal products (yarrow, thyme, rosemary, garlic, mimosa, grapeseed and cranberry) were offered to floor reared chickens up to 42 days of age, and growth performance was measured on days 7, 21 and 42 of the experiment. No treatment effects were detected in the feed intake, weight gain of FCR with any supplemented diet relative to controls ($P>0.05$) at any time during the experiment. In addition, no effects on nutrient digestibility at 21 days of age were observed (Cross *et al.*, 2004a).

Tucker (2002) described the effects of a blend of herbal products (Apex Poultry®, Braes Feed Ingredients, UK; 150mg kg^{-1}) including yarrow, on growth performance and caecal *Clostridia* counts of broilers fed wheat based diets up to 40 days of age. The supplement improved weight gains and FCR equal to that of a traditional AGP (Avilamycin) from 0-20 days of age,

with both additives exceeding ($P<0.05$) the performance levels attained by birds fed unsupplemented control diets. In addition, feeding the herbal supplement reduced caecal *Clostridia* counts at 40 days of age relative to unsupplemented controls. However, Apex Poultry® is a blend of six herbal products, and the effects of yarrow in isolation were not examined.

Proposed mechanisms of action

The antimicrobial activity of yarrow described *in vitro* is likely as a result of its terpene content, but little research has been carried out on its antibacterial mode of action. Terpenes are also found in oregano, which has strong antimicrobial activity *in vitro* (Deans and Svoboda, 1990; Sivropoulou *et al.*, 1996; Skandamis *et al.*, 2000). Indeed several experimental studies document positive growth performance responses in poultry (Waldenstedt, 2000; Demir *et al.*, 2003) and pigs (Kyriakas *et al.*, 1998; Tsinas *et al.*, 1998) following oregano supplementation. The monoterpenes responsible for antimicrobial action in oregano are thymol and carvacrol, which are thought to inhibit pathogenic bacteria by damaging the protein within the cell wall of the bacteria and changing its permeability to phosphate and potassium ions (Lambert *et al.*, 2001). This dissipation of ion gradients leads to an impairment of essential processes within the cell which results in cell death (Heipieper *et al.*, 1991).

There is evidence to suggest that the bitter components present in yarrow may stimulate digestive enzyme secretion and subsequent nutrient digestibility (Chandler, 1982a; Leung and Foster, 1996; Moerman, 1997; Hoffman, 1998; McCartney, 2002). However, the exact component(s) responsible for the bitter action of the herb is not known, although it is likely to be a sesquiterpene lactone (Rodriguez *et al.*, 1976; Picman, 1986).

Yarrow has been given GRAS status, and is approved by the German commission E (Blumenthal, 1998). However, anecdotal evidence suggests that it can be toxic to herbivorous mammals at high intakes (Rodriguez *et al.*, 1976; Picman, 1986). The herb is prized in traditional medicine for its antimicrobial, antifungal and antioxidant properties, and is used to treat a wide range of ailments. In the current study, yarrow is of interest in terms of its antimicrobial properties and its 'bitterness', which may improve nutrient digestibility *in vivo*.

2.4.7 Conclusions and scope of work

In conclusion, this review has highlighted the benefits of AGP in broiler production and investigated the use of alternative options, with the focus on botanical products. Herbs and spices have been exploited by man for millennia for their medicinal properties, and evidence suggests that they have a wide range of effects which could be beneficial in broiler production. As a result of recent interest in this sector, there has been increased scientific output. However, much of the reported experimental work is production orientated, with little investigation into possible mechanisms of action for the botanical compounds tested. In addition, much of the work is proprietary in nature, and as such is commercially sensitive, so little is known about what botanical products have actually been fed. Despite this, botanical products have been shown to have positive growth performance effects in broiler chickens. However, further research is necessary to identify more beneficial botanical products, and to examine them in closer detail in order to elucidate mode of action and identify active compounds within the botanical products.

The current study initially focussed on six selected botanical products: garlic, horseradish, juniper, milk thistle, oregano and yarrow. They have all been assigned 'GRAS' (Generally

Recognised as Safe) status by the Flavour and Extract Manufacturers Association (FEMA) and the Food and Drug Administration (FDA) (Furia and Bellanca, 1975) which implies their use to be safe. The selected products offered a wide range of pharmacological properties, and several mechanisms of action were speculated including antibacterial action *in vivo*, liver protection and digestion stimulation.

The specific objectives of the project were to:

1. identify potentially beneficial botanical extracts through literature review

2. carry out 'screening' experiments to assess the effects of the selected products on growth performance and carcass characteristics of growing broilers

3. select one or two botanical extracts that show positive growth performance effects in the screening experiments, and examine them further in order to elucidate mode of action

3. EXPERIMENTAL

3.1. EXAMINATION OF THE EFFECTS OF INCLUDING BOTANICAL FEED ADDITIVES IN BROILER DIETS

3.1.1 Introduction

The aim of experiments one and two was to investigate the effects of the six selected botanical extracts on the growth performance and carcass characteristics of growing broiler chickens. It was originally decided that experiment two would duplicate experiment one in order to increase the treatment replication. However, due to the unavailability of the oregano product at the commencement of experiment one, treatments varied slightly between the two experiments. Thus experiment one and two are regarded as different experiments, and will be reported and discussed separately.

3.1.2 Specific Objectives

The specific objectives were as follows:

i. To examine the effects of the six selected botanical extracts on broiler weight gain, feed intake and efficiency of feed conversion between 7 and 27 days of age over two periods of determination (7-17 days and 17-27 days).

ii. To examine the effects of feeding the selected botanical extracts on carcass composition, meat yield and organ weights.

iii. To examine the effects of two different levels of inclusion of milk thistle, yarrow and garlic on broiler growth performance and carcass characteristics.

3.1.3 Materials and Methods

The experimental design of these experiments differed only in the botanical extracts tested, therefore plant material and essential oil composition, ration formulation, diet manufacture and animal husbandry were very similar and are reported below for both experiments.

Feed Additives

The botanical feed additives were provided by Braes Feed Ingredients (Table 3.1.1), with the exception of the oregano product which was a commercially available product (All-Natural; Van Beek Global, Orange City, Indianapolis).

Ration Formulation

A wheat/soyabean based ration was formulated to comply with the nutritional specifications recommended for growing broiler chickens outlined by NRC (1994) (Table 3.1.2). The basal diet was supplemented with either a commercially available organic acid blend (Kemira FA2000S), or one of the six selected botanical extracts.

Diet Manufacture

Experimental diets were manufactured at Harper Adams University College. Dietary components were mixed in 25kg batches according to treatment diet specifications (Table 3.1.3). Appropriate concentrations of the premixed experimental ingredients were added to the basal diet and mixed in a horizontal mixer for 3 minutes to ensure a homogenised mixture. The meal was then put through a 'cold' pelleter running at a maximum temperature of 40°C to produce 3mm diameter pellets.

Table 3.1.1 Composition of botanical feed additive premixes

Source herb	Herbal product	Carrier	Additive formulation	($g\ kg^{-1}$)
Garlic	Garlic essential oil from bulb;	Dextrose	Garlic oil	6.0
(*Allium sativum*)	freeze dried alliin extract	Silica	Alliin	1.4
(oil)			Silica	10.0
			Dextrose	982.6
Garlic	Dried and powdered bulb;	Dextrose	Garlic powder	163.2
(*Allium sativum*)	freeze dried alliin extract		Alliin	1.4
(powder)			Dextrose	835.4
Horseradish	Dried and powdered root	Dextrose	Horseradish	60.0
(*Armoracia rusticana*)			Dextrose	940.0
Juniper	Juniper berry essential oil	Dextrose	Juniper oil	4.5
(*Juniperus communis*)		Silica	Silica	15.0
			Dextrose	980.5
Milk Thistle	Dried and powdered seeds	Dextrose	Milk Thistle	1.8
(*Silybum marianum*)	(80% silymarin)		Dextrose	998.2
Yarrow	Dried and powdered herb and	Dextrose	Yarrow	18.0
(*Achillea millefolium*)	flower		Dextrose	982.0

Table 3.1.2 Ingredient composition and calculated analysis of basal diet

Feedingstuff	**Inclusion rate (kg tonne^{-1})**
Wheat	620
Maize gluten meal	40
De-hulled soya bean meal	154
Full fat soya bean meal	120
Soya oil	20
Lysine HCl	3
Methionine	2.5
Limestone	7
Dicalcium phosphate	10
Salt	3.5
Vitamin and trace mineral premix *	20
Total	**1000**
Calculated analysis	
Nutrient	**Concentration (per kg dry matter **)**
Metabolisable Energy	12.9MJ
Crude protein	215g
Crude fibre	30.4g
Lysine	12.3g
Methionine and cystine	9.4g
Calcium	10.1g
Phosphorus	5.7g
Sodium	1.7g

* The vitamin and trace mineral premix contained vitamins and trace elements to meet the requirements specified by NRC (1994). The major components were: phosphorus, 95g/kg; calcium, 219g/kg; sodium, 30g/kg; copper sulphate, 0.5g/kg; selenium, 10mg/kg; retinol acetate, 0.275g/kg; cholecalciferol, 625mg/kg; alpha tocopherol, 2.273g/kg. The product was sourced from Ian Hollows Feed Supplements, Whitchurch, Shropshire.

** Calculated dry matter 875g kg^{-1}

Animal Husbandry

Day-old male Ross 508 broiler chickens were sourced from a proprietary hatchery (Mayfield Chicks, Lancashire). They were placed as one flock in a solid-floored pen covered with wood shavings and offered a commercial broiler starter crumb feed until 5 days of age. The birds were then randomly placed in the experimental cages. Each cage contained four birds and served as a treatment replicate. Cages were accommodated within a climate-controlled environment room. The temperature within the room was gradually reduced from 30°C at day 5 to around 23°C at day 27 replicating standard commercial practice. The lighting regimen was 23 hours light and one hour dark.

A total of 96 cages were arranged in four tiers, with replicates blocked according to tier level. Thus there were two replicates per tier, and eight replicates in total for each of the treatment diets. Each cage measured 0.38m x 0.38m x 0.38m, had a mesh floor (0.025m x 0.025m), and was equipped with two external feeders and a drinking trough. Birds were given *ad libitum* access to feed and water throughout the experiment. Feed was presented in pellet form.

Growth Performance Determination

Growth performance was determined for each treatment replicate. Growth performance was measured over two time periods: 7-17 days of age and 17-27 days of age. Body weight gain and feed consumption were determined for the two growth periods (7-17 days and 17-27 days). In the event of mortality, the weight of the dead and live birds within a cage and feed weight were recorded, and performance results were adjusted accordingly.

Dissections

On the last day of the experiment (27 days of age) two birds per cage (16 birds per dietary treatment) were randomly selected for dissection. Selected birds were sacrificed by cervical dislocation and their viscera were removed. Eviscerated carcass, breast meat, left thigh and bone, left drumstick and bone, skin and feather, abdominal fat (considered to be that fat surrounding the gizzard and intestine, extending within the ischium and surrounding the bursa of Fabricius and cloaca) and liver weights were recorded for each bird.

Statistical Analyses

All growth performance data were analysed as a randomised block design using a general analysis of variance on Genstat 5 (Lawes Agricultural Trust, 1987). Dissection values used were the mean of the two birds from each cage, and were analysed by ANOVA (Genstat 5). Orthogonal contrasts were employed to determine individual performance differences between treatment diets with two levels of herbal additive inclusion. Orthogonal contrasts were made between garlic powder high and low, garlic oil high and low, yarrow high and low and milk thistle high and low treatments diets.

3.1.4 Experimental diets

The effects of six botanical extracts on broiler growth performance and carcass characteristics were compared (Table 3.1.3). Garlic powder, garlic oil, milk thistle and yarrow were thought to have most potential to confer positive effects on broiler growth performance, and so were included at two dietary concentrations (Table 3.1.3). The experimental design described in section 3.1.3. was followed in this experiment.

Table 3.1.3 Experimental diets

Treatment	Additive	Premix inclusion rate ($g\ kg^{-1}$)	Dietary concentration ($mg\ herb\ kg^{-1}\ feed^{1}$)
Control	None	-	-
Acid blend	Acid premix *	10	-
Garlic oil (low)	Garlic oil	5	37
Garlic oil (high)	Garlic oil	10	74
Garlic powder (low)	Garlic powder	5	823
Garlic powder (high)	Garlic powder	10	1646
Juniper	Juniper	10	45
Horseradish	Horseradish	10	600
Milk thistle (low)	Milk thistle	5	9
Milk thistle (high)	Milk thistle	10	18
Yarrow (low)	Yarrow	5	90
Yarrow (high)	Yarrow	10	180

* The acid premix was a commercially available blend (Kemira FA2000S) and contained formic acid, 150g/kg; sodium formate, 160g/kg; lactic acid, 38g/kg; phosphoric acid, 115g/kg; and citric acid, 10g/kg carried on a diatomaceous earth. The product was sourced from Ian Hollows Feed Supplements, Whitchurch, Shropshire.

3.1.5 Results

Mortality was low during the experiment (1.1%) and was unaffected by dietary treatment. Mean growth rates of birds in this experiment were 11.2% higher than Ross 508 commercial broiler performance targets over the same growing period (7-27d) (Ross Breeders, 1999). Feed intake was also higher (15.1%) which resulted in a small reduction of FCE (0.05 points lower in comparison with Ross targets). On average, birds reached 1.4kg live weight at 27 days of age, four days ahead of target.

No statistically significant treatment differences in weight gain, feed intake or FCE were observed during this experiment (tables 3.1.4, 3.1.5 and 3.1.6 respectively). During the earlier growth period (7-17 days), birds fed dietary treatments containing the higher levels of both garlic and yarrow had numerically superior weight gains compared to controls (Table 3.1.4), but this did not reach statistical significance (P=0.083) and was not seen in the second growth period.

Orthogonal contrasts made between treatment diets containing different concentrations of the same herbal feed additive showed that birds fed the higher rate of yarrow tended (P=0.091) to have higher weight gains (Table 3.1.4) and feed intake (Table 3.1.5) than those fed the lower rate during the initial growing period. Birds fed higher levels of garlic powder tended (P=0.089) to grow faster than those fed the lower rate throughout the experimental period (Table 3.1.4) resulting in better FCE values (P<0.05, 7-27d; P=0.063, 17-27d) (Table 3.1.6). However, orthogonal contrasts did not reveal any additive effects of feeding garlic oil.

Table 3.1.4 Effect of plant extracts on individual bird weight gain (7-27d)

Treatment Diet	Weight Gain (g day^{-1})		
	7-27d	7-17d	17-27d
Control	62.8	45.9	79.5
Acid Blend	62.3	46.2	78.3
Milk Thistle (low)	61.7	44.9	78.5
Milk Thistle (high)	62.0	43.2	80.9
Yarrow (low)	60.8	44.0	77.6
Yarrow (high)	60.8	46.7	74.9
Garlic oil (low)	62.1	45.8	78.4
Garlic oil (high)	61.3	43.2	79.5
Garlic powder (low)	60.8	44.9	76.8
Garlic powder (high)	64.3	46.7	81.8
Juniper	63.1	43.9	82.8
Horseradish	63.2	44.7	81.7
Grand mean	62.1	45.0	79.2
SEM	0.17	0.14	0.31
CV (%)	5.5	6.1	7.9
P	NS	0.083	NS
Contrast statements		*Probability levels of contrast*	
Milk thistle: low vs high	NS	NS	NS
Yarrow: low vs high	NS	0.091	NS
Garlic oil: low vs high	NS	NS	NS
Garlic powder: low vs high	0.089	NS	NS

NS=*P*>0.1

Table 3.1.5 Effect of plant extracts on individual feed intake (7-27d)

Treatment Diet	Feed Intake (g day^{-1})		
	7-27d	7-17d	17-27d
Control	97.2	66.6	127.7
Acid Blend	99.5	69.7	129.3
Milk Thistle (low)	96.9	68.9	124.8
Milk Thistle (high)	96.3	64.9	127.6
Yarrow (low)	98.2	66.1	130.4
Yarrow (high)	97.3	67.2	131.5
Garlic oil (low)	100.9	66.5	135.2
Garlic oil (high)	98.8	65.5	132.0
Garlic powder (low)	101.3	67.1	135.5
Garlic powder (high)	99.5	66.9	132.0
Juniper	98.9	65.9	131.8
Horseradish	99.1	65.2	133.0
Grand mean	98.6	66.7	130.9
SEM	0.31	0.21	0.50
CV (%)	6.3	6.2	7.6
P	NS	NS	NS
Contrast statements		*Probability levels of contrast*	
Milk thistle: low vs high	NS	0.071	NS
Yarrow: low vs high	NS	0.091	NS
Garlic oil: low vs high	NS	NS	NS
Garlic powder: low vs high	NS	NS	NS

NS=*P*>0.1

Table 3.1.6 Effect of plant extracts on feed conversion efficiency (g gain: g feed)

	FCE		
Treatment Diet	**7-27d**	**7-17d**	**17-27d**
Control	0.646	0.692	0.663
Acid Blend	0.626	0.664	0.607
Milk Thistle (low)	0.640	0.653	0.633
Milk Thistle (high)	0.645	0.666	0.635
Yarrow (low)	0.621	0.668	0.598
Yarrow (high)	0.627	0.696	0.571
Garlic oil (low)	0.616	0.688	0.581
Garlic oil (high)	0.621	0.661	0.601
Garlic powder (low)	0.602	0.670	0.571
Garlic powder (high)	0.646	0.696	0.621
Juniper	0.639	0.668	0.625
Horseradish	0.640	0.686	0.619
Grand mean	0.631	0.676	0.607
SEM	0.0195	0.0207	0.0259
CV (%)	6.2	6.1	8.5
P	NS	NS	NS
Contrast statements		*Probability levels of contrast*	
Milk thistle: low vs high	NS	NS	NS
Yarrow: low vs high	NS	NS	NS
Garlic oil: low vs high	NS	NS	NS
Garlic powder: low vs high	**0.034**	NS	0.063

NS=*P*>0.1

No significant (P>0.05) treatment differences in carcass composition were observed (Table 3.1.7). Orthogonal contrasts made between treatment diets with different inclusion levels of the same herbal additive showed that birds fed higher levels of milk thistle, garlic oil and garlic powder had a greater proportional drumstick weights than their lower level counterparts. However, there were no statistical differences in proportions of total leg meat or total meat. Although eviscerated carcass weights were significantly higher for birds fed diets containing higher levels of garlic powder, body weight was also higher ($P<0.05$; data not shown), and when eviscerated carcass weight is expressed as a proportion of body weight, no statistical significance between garlic powder treatments is seen ($P>0.05$; data not shown). Birds fed the lower level of yarrow had proportionally higher fat pad weights than those fed the higher level (1.86 and 1.31% of eviscerated carcass for low and high level yarrow respectively; $P<0.05$).

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Table 3.1.7 Effect of dietary treatment on carcass composition (values expressed as a percentage of eviscerated carcass weight)

Treatment Diet	Eviscerated carcass weight (g)	Breast meat	Drumstick and bone	Thigh and bone	Total leg meat	Total meat	Liver	Fat pad
Control	833.0	26.82	15.04	19.13	34.17	60.98	4.02	1.63
Acid Blend	845.0	25.51	14.83	19.00	33.83	59.35	4.17	1.65
Milk Thistle (low)	849.9	26.10	14.58	18.43	33.01	59.11	3.81	1.71
Milk Thistle (high)	825.0	26.05	15.46	18.38	33.84	59.89	3.94	1.84
Yarrow (low)	522.4	25.79	15.17	18.24	33.41	59.21	4.14	1.86
Yarrow (high)	839.9	25.15	14.91	18.57	33.47	58.62	3.99	1.31
Garlic oil (low)	871.2	26.42	14.21	19.00	33.21	59.62	3.91	1.44
Garlic oil (high)	827.1	26.50	15.18	19.13	34.31	60.80	4.01	1.77
Garlic powder (low)	823.7	25.56	14.46	18.63	33.09	58.65	4.09	1.83
Garlic powder (high)	886.5	26.22	15.37	18.62	33.99	60.22	3.96	1.83
Juniper	827.6	26.29	15.02	19.27	34.29	60.58	3.94	1.71
Horseradish	874.8	26.11	14.67	18.08	32.75	58.86	3.85	1.68
Grand mean	843.8	26.04	14.91	18.71	33.61	59.66	3.99	1.69
SEM	28.24	0.717	0.464	0.697	0.764	0.995	0.233	0.232
CV (%)	9.5	7.8	8.8	10.5	6.4	4.7	16.6	38.9
P	NS	NS	NS	NS	NS	NS	NS	NS
Contrast statements			*Probability levels of contrast*					
Milk thistle: low vs high	NS	NS	**0.037**	NS	NS	NS	NS	NS
Yarrow: low vs high	NS	NS	NS	NS	NS	NS	NS	**0.020**
Garlic oil: low vs high	NS	NS	**0.038**	NS	NS	NS	NS	NS
Garlic powder: low vs high	**0.027**	NS	0.051	NS	NS	NS	NS	NS

NS=*P*>0.1

3.1.6 Discussion

This section represents a preliminary discussion of experiment one. The results will also be discussed in relation to the findings of experiment two (section 3.1.8). It was expected that the inclusion of herbal feed additives in the diets of growing broiler chickens would stimulate growth performance on par with AGP, but no treatment differences were observed. However, the efficacy of AGP is highly variable (Rosen, 1995; Gustafson, 1997; Johnston, 2001), and the magnitude of bird growth response to AGP is dependant on the environment in which the birds are reared, with higher responses seen in birds reared in a poorer environment (Dafwang *et al.*, 1987; Rosen, 1995; Thomke and Elwinger, 1998). Environmental conditions in the present study were clean and hygienic.

A major factor contributing to the lack of statistical differences between treatment diets was the high quality of this particular batch of chickens. The chickens had high initial start weights and grew faster than the performance targets set out by Ross Breeders. It is therefore not surprising that no treatment differences were seen: the chickens were growing at a rate very close their genetic potential, so any possible changes in performance as a result of feeding the botanical extracts would have been very small. It can be calculated (WinEpiscope, 1996) that higher replication would be required in order to detect significant differences between these growth performance data: for example detection of significant improvements in growth performance of 5 and 3% would require 9 and 22 replicates respectively. An improvement of 2-3% would still be commercially important, so even though no statistical differences were detected in this experiment, some treatment diets, although not statistically proven, may have improved the performance of growing broiler chickens.

Although no treatment diet significantly influenced growth performance relative to controls, orthogonal contrasts made between bids fed differing levels of garlic powder showed that birds fed the higher inclusion level had improved feed conversion efficiency ($P<0.05$) relative to those fed the lower rate. However, these results are in conflict with previous findings. Horton and workers (1991b) studied the effects of adding garlic (100, 1000 and 10 000 mg kg^{-1}) to the diets of growing broiler chickens (7-28d) and found that garlic supplementation improved weight gains ($P<0.05$) relative to controls, but that gains decreased as garlic concentration increased ($P<0.05$). These results are in agreement with those of Qureshi *et al.* (1983a) who reported depressed feed consumption and lower gains with increasing levels of garlic. However, it should be noted that Qureshi *et al.* (1983a) and Horton *et al.* (1991b) fed considerably higher levels of garlic (up to 80 000mg kg^{-1}). In the present experiment levels of garlic fed were lower, but were considered to be sufficient on the basis of previous *in vitro* studies (Ankri and Mirelman, 1999; Harris *et al.*, 2001; Ross *et al.*, 2001; Yin *et al.*, 2002).

3.1.7 Experiment 2

It was originally decided that experiment two would duplicate experiment one in order to increase the treatment replication. However, due to the unavailability of the oregano product at the commencement of experiment one, treatments varied slightly between the two experiments. In the present experiment, extracts of garlic, juniper, horseradish, milk thistle, yarrow and oregano were compared. Again, three of the selected extracts (garlic, milk thistle and yarrow) were considered to have most potential to confer positive effects on broiler growth performance, and so were included at two dietary concentrations (Table 3.1.8). The experimental design outlined in section 3.1.3. was followed in this experiment.

Table 3.1.8 Experimental diets

Treatment	Additive	Premix inclusion rate ($g\ kg^{-1}$)	Dietary concentration ($mg\ kg^{-1}$ herb)
Control	None	-	-
Acid blend	Acid premix *	10	-
Garlic powder (low)	Garlic powder	5	823
Garlic powder (high)	Garlic powder	10	1646
Juniper	Juniper	10	45
Horseradish	Horseradish	10	600
Milk thistle (low)	Milk thistle	5	9
Milk thistle (high)	Milk thistle	10	18
Oregano	Oregano	10	N/A
Yarrow (low)	Yarrow	5	90
Yarrow (high)	Yarrow	10	180

* The acid premix was a commercially available blend (Kemira FA2000S) and contained formic acid, 150g/kg; sodium formate, 160g/kg; lactic acid, 38g/kg; phosphoric acid, 115g/kg; and citric acid, 10g/kg carried on a diatomaceous earth. The product was sourced from Ian Hollows Feed Supplements, Whitchurch, Shropshire.

3.1.8 Results

Mortality was low (1.3%) and was unaffected by treatment diet ($P>0.05$). There was a relatively large range of weight gains (14.3%), but a small range in feed intakes (7.2%) between treatments during the whole growth period (Tables 3.1.9 and 3.1.10). However, these differences were not statistically significant ($P>0.05$). The mean weight gain of birds over the experimental period (7-27 days) was 7.3% lower than Ross targets for male Ross 508 chickens. However, they consumed 1.2% more food, which was reflected in the lower FCE values seen (Grand mean 0.61 compared with Ross targets of 0.67 over the same growing period). Bird live weight was, on average, 2 days below Ross target body weight at day 5. However, average body weight was still 2 days below target on day 27 indicating that the birds had maintained expected growth rates throughout the experiment.

Again, no treatment differences in FCE were observed between 7 and 27 days of age (Table 3.1.11), but birds fed the garlic powder and yarrow high treatment diets tended ($P=0.076$) to convert feed more efficiently than birds fed other treatment diets. No statistical trends were seen over the initial growing period (7-17d), though FCE values for birds fed garlic powder and yarrow high were numerically superior. During the second growth period, this pattern reached statistical significance ($P<0.05$). Orthogonal contrasts made between birds fed yarrow treatment diets revealed that efficiency of feed conversion was superior in birds fed the higher level (17-27d, $P<0.05$; 7-27d, $P=0.064$). Weight gains were similar for both yarrow treatment diets (Table 3.1.9), but birds fed diets containing the lower rate ate (6.8%) more feed overall (Table 3.1.10), specifically during the second growth period (118.9 *vs.* 104.4g day^{-1} for low and high respectively, $P<0.05$). There were no statistical differences in growth performance

between birds fed the two garlic powder treatment diets, although the performance of birds fed the higher level was numerically better than those fed the lower level.

During the second growth period birds fed diets supplemented with the lower level of milk thistle had significantly depressed FCE values in comparison with birds fed the other treatment diets. Comparisons made between birds fed milk thistle diets revealed that FCE tended to be better in birds fed the higher level (17-27d, P=0.054; 7-27d, P=0.082) because of higher weight gains (17-27d, P<0.01; 7-27d, P<0.05).

Table 3.1.9 Effect of plant extracts on individual bird weight gain (7-27d)

	Weight Gain (g day^{-1})		
Treatment Diet	**7-27d**	**7-17d**	**17-27d**
Control	51.1	36.3	65.8
Acid Blend	51.9	36.3	67.6
Milk Thistle (low)	46.1	34.3	57.9
Milk Thistle (high)	52.6	36.0	69.3
Yarrow (low)	52.0	36.2	67.9
Yarrow (high)	52.3	36.9	67.7
Garlic powder (low)	51.1	32.9	69.4
Garlic powder (high)	53.8	35.8	71.9
Oregano	52.0	35.6	68.5
Juniper	51.7	36.9	66.5
Horseradish	50.2	33.0	67.4
Grand mean	51.4	35.5	67.4
SEM	2.13	1.54	3.34
CV (%)	9.6	10.0	11.5
P	NS	NS	NS
Contrast statements		*Probability levels of contrast*	
Milk thistle: low vs high	**0.010**	NS	**0.004**
Yarrow: low vs high	NS	NS	NS
Garlic powder: low vs high	NS	NS	NS

NS=P>0.1

Table 3.1.10 Effect of plant extracts on individual feed intake (7-27d)

	Feed Intake (g day^{-1})		
Treatment Diet	**7-27d**	**7-17d**	**17-27d**
Control	84.9	57.0	112.8
Acid Blend	88.1	57.2	119.0
Milk Thistle (low)	81.9	55.5	108.2
Milk Thistle (high)	87.2	57.8	116.6
Yarrow (low)	86.9	54.9	118.9
Yarrow (high)	81.0	57.5	104.4
Garlic powder (low)	80.9	54.8	106.9
Garlic powder (high)	84.2	56.8	111.6
Oregano	85.1	55.5	114.7
Juniper	84.7	56.9	112.5
Horseradish	84.4	55.1	113.6
Grand mean	84.5	56.2	112.8
SEM	3.13	1.62	5.45
CV (%)	8.6	6.6	11.2
P	NS	NS	NS
Contrast statements		*Probability levels of contrast*	
Milk thistle: low vs high	NS	NS	NS
Yarrow: low vs high	NS	NS	**0.024**
Garlic powder: low vs high	NS	NS	NS

NS=*P*>0.1

Table 3.1.11 Effect of plant extracts on feed conversion efficiency (g gain: g feed)

	FCE		
Treatment Diet	**7-27d**	**7-17d**	**17-27d**
Control	0.599	0.638	0.581
Acid Blend	0.590	0.634	0.571
Milk Thistle (low)	0.562	0.618	0.531
Milk Thistle (high)	0.608	0.625	0.603
Yarrow (low)	0.600	0.659	0.573
Yarrow (high)	0.649	0.640	0.657
Garlic powder (low)	0.633	0.599	0.652
Garlic powder (high)	0.642	0.631	0.649
Oregano	0.612	0.641	0.599
Juniper	0.614	0.650	0.598
Horseradish	0.595	0.599	0.594
Grand mean	0.610	0.631	0.600
SEM	0.0227	0.0240	0.0314
CV (%)	8.6	8.8	12.1
P	0.076	NS	**0.021**
Contrast statements		*Probability levels of contrast*	
Milk thistle: low vs high	0.082	NS	0.054
Yarrow: low vs high	0.064	NS	**0.024**
Garlic powder: low vs high	NS	NS	NS

NS=*P*>0.1

Effects of dietary treatment on carcass composition are presented in table 3.1.12. Relative to controls, birds fed diets containing the acid blend and the lower level of garlic powder had proportionally higher ($P<0.05$) drumstick and bone weights, and tended ($P=0.98$) to have proportionally more total leg meat, but total meat yield was unaffected ($P>0.05$). Orthogonal contrasts made between birds fed garlic powder treatment diets showed that birds fed the lower rate tended to have heavier proportional drumstick and bone weights (15.23 *vs.* 14.52% of eviscerated carcass weight for low and high respectively; $P=0.096$), but deposited less tissue as breast muscle than birds fed the higher level (22.78 *vs.* 24.54% for low and high respectively; $P<0.05$). In addition, proportional fat pad weights tended to be higher ($P=0.087$) in birds fed the lower levels.

Analysis of carcass data revealed that liver weights were unaffected by treatment diet, although birds fed the control treatment diet and the yarrow high treatment diet tended ($P=0.073$) to have heavier proportional liver weights, whereas birds fed the garlic high diet tended ($P=0.073$) to have lower proportional liver weights. Orthogonal contrasts showed that birds fed the garlic powder high diets had lower liver weights than those fed the lower rate ($P<0.01$), but that birds fed the higher rate of yarrow had heavier liver weights than those fed the lower rate ($P<0.05$).

Table 3.1.12 Effect of dietary treatment on carcass composition (values expressed as a percentage of eviscerated carcass weight)

Treatment Diet	Eviscerated carcass weight (g)	Breast meat	Drumstick and bone	Thigh and bone	Total leg meat	Total meat	Liver	Fat pad
Control	728.4	24.15	14.16	17.67	31.82	55.64	4.53	1.32
Acid Blend	744.8	22.93	15.27	17.80	33.07	55.99	4.26	1.65
Milk Thistle (low)	702.6	23.93	14.31	17.05	31.36	55.29	4.47	1.36
Milk Thistle (high)	734.8	24.64	14.01	17.83	31.84	56.48	4.30	1.54
Yarrow (low)	731.4	23.44	14.99	16.85	31.85	55.29	4.25	1.80
Yarrow (high)	698.2	24.27	14.45	17.58	32.03	56.30	4.62	1.56
Garlic powder (low)	714.9	22.78	15.23	17.96	33.19	55.97	4.64	1.51
Garlic powder (high)	730.9	24.54	14.52	17.57	32.09	56.63	4.09	1.19
Oregano	739.9	24.20	14.38	17.11	31.48	55.68	4.20	1.45
Juniper	716.6	23.93	14.51	17.49	31.99	55.93	4.50	1.34
Horseradish	747.1	24.16	14.01	17.14	31.14	55.30	4.35	1.48
Grand mean	727.7	23.93	14.52	17.43	31.95	55.85	4.37	1.47
SEM	25.17	0.762	0.369	0.493	0.625	0.972	0.175	0.163
CV (%)	11.3	10.4	8.3	9.2	6.4	5.7	13.1	36.2
P	NS	NS	**0.018**	NS	0.098	NS	0.073	0.097
Contrast statements				*Probability levels of contrast*				
Milk thistle: low vs high	NS	NS	NS	NS	NS	NS	NS	NS
Yarrow: low vs high	NS	NS	NS	NS	NS	NS	**0.029**	NS
Garlic powder: low vs high	NS	**0.047**	0.096	NS	NS	NS	**0.007**	0.087

NS=*P*>0.1

3.1.9 Discussion

The lack of growth-promoting action is surprising given the reported *in vitro* antimicrobial activity of oregano (Piccaglia *et al.*, 1993; Dorman and Deans, 2000), juniper (Hammer *et al.*, 1999; Chao *et al.*, 2000) and horseradish (Delaquis and Mazza, 1995; Dorman and Deans, 2000). Although garlic and yarrow supplementation did not affect weight gain or feed intake, chickens fed the higher rates of these two additives tended to have superior feed conversion efficiency throughout the experimental period (P=0.076), principally during the latter growth stage (P<0.05). This may be attributable to the *in vitro* antimicrobial activity reported for garlic (Arora and Kaur, 1999; Harris *et al.*, 2001) and yarrow (Bishop, 1951; Candan *et al.*, 2003).

Cross *et al.* (2004a) fed garlic (1000mg kg^{-1}) to caged male broilers up to 21 days of age. Although weight gains and feed intake were numerically superior to controls, no statistically significant trends were observed which is consistent with the findings of this experiment. However, in this experiment birds fed the high inclusion garlic powder treatment diets showed improved FCE (17-27, P<0.05; 7-27. P=0.076) relative to controls. Horton *et al.* (1991b) also highlighted the beneficial effects of garlic on broiler growth performance over the same time period, where chickens had higher weight gains (P<0.05) than birds fed control treatment diets.

Orthogonal contrasts made between birds fed garlic powder treatment diets showed that those fed the higher rate deposited proportionally more breast meat than those fed the lower rate (P<0.05). Demir *et al.* (2003) demonstrated that garlic supplementation (1000mg kg^{-1}) resulted in shallower crypts (P<0.05) relative to AGP fed broilers (0-42 days of age). A large crypt

indicates rapid tissue turnover and a high demand for new tissue, thus an increase in nutrient requirement for maintenance (Savage *et al.*, 1997). Energy conserved through reduced turnover of epithelial cells is then available for lean tissue synthesis, which, assuming an additive effect of garlic on crypt depth, may explain the higher yields of breast meat seen in birds fed treatment diets containing the higher level of garlic powder. However, it is more likely that the higher breast meat yields noted in birds fed diets containing the high inclusion garlic powder are attributable to the better growth performance of these birds, as faster growth rates result in greater deposition of breast meat (Rose, 1997).

Improvements in FCE seen in birds fed yarrow high treatment diets are consistent with the findings of Cross *et al.* (2002) who fed yarrow to caged broilers up to 28 days of age. These authors hypothesised that the effects seen were a result of beneficial moderation of intestinal microflora. Orthogonal contrasts revealed that birds fed the yarrow high treatment diets had superior FCE to those fed the yarrow low treatment diets during the second growth phase because they ate less feed ($P<0.05$) but attained similar rates of weight gain (67.92 *vs.* 67.70g day^{-1} for yarrow low and yarrow high birds respectively). Birds fed the lower rate of yarrow had a greater share of abdominal fat than their higher rate conspecifics, which is consistent with results from the first experiment (Experiment 1: 1.86 *vs.* 1.31% carcass weight for low and high respectively, $P<0.05$; Experiment 2: 1.80 *vs.* 1.56% for low and high respectively, $P>0.05$). Jamroz *et al.* (2003) also observed significant reductions of abdominal fat deposited in birds fed higher inclusion rates of a herbal supplement in comparison with birds fed a lower inclusion rate of the same supplement, and ranges in these screening experiments are consistent with their reported values. In view of this observation and the similar rates of daily gain, it appears that the birds fed the higher level of yarrow have utilised nutrients and energy to deposit body protein rather than body fat, but the mechanism for this is unclear. Herbs have

been shown to reduce blood cholesterol levels in rats (Cheng *et al.*, 2004) and humans (Thompson-Coon and Ernst, 2003), and significant dose-related reductions in cholesterol in response to dietary garlic supplementation have been observed in chickens (Qureshi *et al.*, 1983a; Qureshi *et al.*, 1983b; Horton *et al.*, 1991b). This suggests an involvement in fat metabolism in animals, but nothing in the literature could be found on the effect of yarrow on fat metabolism.

Considerable research has focussed on the inclusion of oregano as a growth promoter in monogastrics. The majority of experiments reported in the literature pertaining to the use of oregano in pigs demonstrate significant positive growth responses. In poultry however, the response is more variable, with observed effects being either positive (Waldenstedt, 2000; Demir *et al.*, 2003) or non-significant (Botsoglou *et al.*, 2002; Cross *et al.*, 2002; Lee *et al.*, 2003a). It is possible that the faster intestinal transit time and smaller volumes of digesta increase the variability in chickens compared with pigs. Cross *et al.* (2002) found no statistical growth improvement in broiler chickens fed oregano up to 28 days of age, which is in agreement with the present study. However, Waldenstadt (2000) and Demir *et al.* (2003) reported significant ($P<0.05$) improvements in live weight gain from days 13-48 and 0-42 respectively, suggesting that any possible beneficial effects on growth performance may only be seen in the later stages of broiler growth.

Despite strong antibacterial effects demonstrated *in vitro*, both juniper and horseradish supplementation failed to elicit any growth response in this experiment. It may be that any positive growth effects were too small to be detected, or that the antimicrobial effects *in vitro* were not as pronounced *in vivo*. Indeed work by Ross *et al.* (2001) has demonstrated that the antimicrobial activity of garlic oil (but not garlic powder) is markedly reduced in the presence

of cysteine, which suggests that the effects of essential oils seen *in vitro* may be reduced in the enteric environment. In addition, work by Cross *et al.* (2002) has demonstrated large discrepancies in growth response between herbal additives when fed as herbs or their volatile oil constituents. The lack of response to horseradish was surprising given the plethora of positive responses observed when other pungent substances have been fed (Horton *et al.*, 1991b; Piva *et al.*, 1991; Ilsley *et al.*, 2002; Jamroz *et al.*, 2003; Samarasinghe *et al.*, 2003). However, sinigrin, the principal antibiotic component found in horseradish, is highly volatile and rapidly decomposes into inactive compounds in the presence of myrosin (Brabban and Edwards, 1995; Shofran *et al.*, 1998) which may explain the lack of response *in vivo*.

Experiments using Livol©, a commercial product containing milk thistle, have shown that growth performance of birds fed the additive did not vary from that of birds fed AGP supplemented diets (Dobretsberger *et al.*, 1997). However, in contrast to the present experiment, these birds were housed in commercial conditions, which are likely to be more challenging to the birds and therefore more likely to show a response, as discussed previously. In the present study, birds fed milk thistle treatment diets did not outperform control fed birds, although orthogonal contrasts detected differences between the two levels of inclusion. Birds fed the higher level had better growth rates overall ($P<0.05$), specifically during the second growth period ($P<0.01$). This was not accompanied by an increase in feed intake ($P>0.05$) indicating that the higher weight gains must be as a result of greater feed conversion efficiency. Flavonoids are purported to stimulate bile salt production (McCartney, 2002) that have a major role in fat digestion (Leeson and Summers, 2001). In experiments with rats, milk thistle had an additive effect on bile salt secretion (Crocenzi *et al.*, 2000). It is plausible that this additive effect on bile salt secretion and subsequent improved efficiency of fat digestion

may account for the discrepancies seen between birds fed the two levels of milk thistle. However, no additive effects were seen in the first experiment.

Dietary treatment tended to affect proportional liver weights, but this was not statistically proven (P=0.073) which is consistent with the findings of other similar studies (Jamroz *et al.*, 2003; Lee *et al.*, 2003b; Hernandez *et al.*, 2004; Jang *et al.*, 2004). In contrast, Lee *et al.* (2003a) found that birds fed diets supplemented with thymol (10 mg kg^{-1}) up to 21 days of age had heavier liver weights than control fed birds (P<0.05), but that the effect had disappeared by 40 days of age.

In conclusion, birds fed the garlic powder high and yarrow high diets showed some positive growth performance effects, even in a clean environment. These botanical products therefore merit further investigation.

3.1.8 General discussion of screening experiments

Weight gain and feed intake were not influenced by dietary treatment throughout either experiment, which was surprising given the range of pharmacological properties reported for the chosen herbal additives. It is interesting to note that the 'positive control' (acid blend) failed to elicit any growth performance effects during either experiment. However, this is consistent with Gollnisch's (2001) work where pigs failed to respond to treatment diets containing either oregano or a traditional AGP. A model (Cole, 1991) of porcine growth response to probiotics may explain this inconsistency. The model suggests that the magnitude of response to probiotics varies according to performance. Consequently, little or no response can be expected at high performances, but the response may increase with substandard performances. This hypothesis is certainly consistent with previous studies (Coates *et al.*, 1951; Hill *et al.*, 1952; Forbes and Park, 1959) where well-nourished healthy chicks responded less to antibiotic supplements when they were housed in a carefully cleaned and disinfected environment.

In retrospect there may have been insufficient microbial challenge against these birds for beneficial effects to be detected. It has been shown previously that cage environments offer the cleanest option in poultry production (Hoglund *et al.*, 1995; Permin *et al.*, 1999; Willis *et al.*, 2002). In future experiments it may prove beneficial to raise birds in an environment more representative of a commercial situation, for example on litter which provides a substrate for pathogenic bacterial growth (Pope and Cherry, 2000).

These initial experiments were conducted primarily for screening purposes, and focussed on testing a wide range of herbs. It is concluded tentatively that dietary inclusion of both garlic

powder and yarrow may improve broiler growth performance. The emphasis of further work will therefore be on garlic powder and yarrow. Orthogonal contrasts between inclusion levels suggest that the higher dietary concentration of both additives have a greater effect on growth performance than the lower rates. The question then arises as to the possible underlying mechanisms of action. It is plausible that growth effects seen may be attributed to the *in vitro* antimicrobial activity reported for garlic (Arora and Kaur, 1999; Harris *et al.*, 2001) and yarrow (Bishop, 1951; Candan *et al.*, 2003). It is therefore hypothesised that both garlic and yarrow confer their beneficial effects on broiler growth performance by reducing pathogenic bacterial species and/or maintaining the beneficial species in order to maintain a stable and beneficial flora. This is certainly consistent with putative mechanisms of action for AGP (Coates *et al.*, 1963; Fuller *et al.*, 1979; Engberg *et al.*, 2000), organic acids (Hyden, 2000; Hillman, 2001), enzymes (Bedford, 2000; Engberg and Petersen, 2001) and probiotics (Vanbelle *et al.*, 1990; Collins and Gibson, 1999).

3.2. FURTHER EXAMINATION OF GARLIC POWDER AND YARROW

3.2.1 Introduction

Results from the previous experiments indicate that both garlic powder and yarrow improved the feed conversion efficiency of caged broiler chickens when fed as part of a nutritionally complete diet. However, improvements in growth performance were somewhat small. It was hypothesised that the primary mode of action of both garlic powder and yarrow was related to their antimicrobial properties, so it was assumed that the small growth responses seen were associated with the high level of environmental hygiene of the experimental conditions. This is in agreement with numerous field studies (Anonymous, 1997; Bassett, 2000; Langhout, 2000; Kamel, 2001a; Kamel, 2001b) and controlled experiments (de Freitas *et al.*, 2001; Botsoglou *et al.*, 2002; Cross *et al.*, 2002; Lee, 2003a; Cross *et al.*, 2004a) in which herbs with antimicrobial activity have been fed, which conclude that the effects of dietary herbal supplementation on broiler growth performance may be masked when chickens are reared in a clean environment. Thus in the present experiments, birds were reared on shavings in floor pens offering a situation more typical of commercial conditions. In addition, a proportion of 'old' litter was added to each experimental pen to increase microbial challenge.

In the previous experiment, both garlic powder and yarrow improved FCE, but only during the latter growth stage (17-27d), which is consistent with the findings of Cross *et al.* (2002). Therefore in the present experiments, the effects of herbal supplementation over two feeding periods were examined. Birds were offered one of two diets (control or herb supplemented) over two feeding periods (starter, 0-18d; grower, 18-36d) in a 2x2 factorial design, thus there were four experimental diets in total.

Many herbs, including garlic and yarrow, have demonstrated antibacterial activity *in vitro*. It was hypothesised that the ability of garlic and yarrow to control pathogenic bacterial species *in vitro* may be seen *in vivo*, changing the gut microflora in a positive way and thus explaining the beneficial growth performance effects seen when these herbs are fed. Although reports of antibacterial activity *in vitro* are numerous and congruent, findings of *in vivo* broiler experiments are inconsistent, with herbs either having a positive impact on gut microflora (Tucker, 2002; Jamroz *et al.*, 2003; Samarasinghe *et al.*, 2003) or no effect (Cross *et al.*, 2001; Jang *et al.*, 2004). Positive modulation of gut microflora has also been shown to improve litter quality by decreasing excreta water concentrations (Elwinger *et al.*, 1996). Wet litter causes carcass downgrades at the slaughterhouse due to the increased incidence of breast blisters, skin burns, bruising and condemnations, and also to increase ammonia production in broiler houses.

3.2.2 Specific Objectives

The following chapter describes two experiments performed at two different times feeding two different herbal products (garlic powder and yarrow). However, the materials and methods were the same for both experiments and thus are described only once.

The overall objectives of the following experiments were to examine the effects of both garlic powder and yarrow over two feeding periods in conditions representative of a commercial situation. In the event of a positive growth response to either herbal supplement, the hypothesis that their beneficial effects are mediated through moderation of the gut microflora was also tested. The specific objectives each of these two experiments were to investigate the effects of herbal supplementation on:

i. broiler growth performance over two feeding periods under commercial conditions

ii. the prevalence of coccidiosis (*Eimeria tenella* and *Eimeria necatrix*)

iii. litter quality

iv. caecal microflora (in the event of a positive growth response)

3.2.3 Materials and Methods

Feed Additives

Braes Feed Ingredients (Chester, UK) provided the herbal premixes. Dietary concentrations of garlic powder and yarrow were equivalent to those used in previous experiments, but the premixes were formulated to be incorporated at 5g kg^{-1} rather than the 10g kg^{-1} used previously. The garlic powder premix contained 16.32g kg^{-1} dried garlic powder and 140mg kg^{-1} purified alliin extract w/w on a dextrose carrier.

Ration Formulation

Broilers were fed one of two treatment diets during two feeding periods in a 2x2 factorial design (Table 3.2.1). Feeding periods were 0-18 days (starter) and 18-36 days (grower). Nutritionally complete starter and grower feeds were formulated to comply with NRC (1994) recommendations (Table 3.2.2). Basal diets were supplemented with 5kg $tonne^{-1}$ of either the herbal additive or dextrose (control). No additional anticoccidials, antimicrobials or enzymes were added to the diets. Feed, presented in pellet form, and water were available *ad libitum*.

Table 3.2.1 Dietary treatments

	Additive	
Treatment Diet	**Starter (0-18 days)**	**Grower (18-36 days)**
- - (control)	Control	Control
- +	Control	Garlic
+ -	Garlic	Control
+ +	Garlic	Garlic

Table 3.2.2 Ingredient composition and calculated analysis of basal diets

Feedingstuff	Inclusion rate (kg tonne^{-1})	
	Starter	**Grower**
Wheat	615	600
De-hulled soya bean meal	133	140
Full fat soya bean meal	133	190
Fishmeal	50	-
Soya oil	25	25
Lysine HCl	1.5	1.5
Methionine	2.5	2.5
Limestone	7	7
Dicalcium phosphate	5	5
Salt	3	4
Vitamin and trace mineral premix [1]	20	20
Herbal product/dextrose control	5	5
Total	**1000**	**1000**
Calculated analysis		
Nutrient	**Concentration (per kg dry matter [2])**	
Metabolisable Energy	13.0 MJ	13.1MJ
Crude protein	216g	205g
Crude fibre	29.5g	32.8g
Lysine	12.9g	11.88g
Methionine and cystine	9.3g	8.7g
Calcium	10.8g	9.04g
Phosphorus	5.8g	4.9g
Sodium	1.9g	1.9g

[1] The vitamin and trace mineral premix contained vitamins and trace elements to meet the requirements specified by NRC (1994). The major components were: phosphorus, 95g/kg; calcium, 219g/kg; sodium, 30g/kg; copper sulphate, 0.5g/kg; selenium, 10mg/kg; retinol acetate, 0.275g/kg; cholecalciferol, 625mg/kg; alpha tocopherol, 2.273g/kg. The product was sourced from Ian Hollows Feed Supplements, Whitchurch, Shropshire.

[2] Calculated dry matter: starter 878g kg^{-1}; grower 876g kg^{-1}

Diet Manufacture

Feed was manufactured at Harper Adams University College. Experimental diets were mixed in 25kg batches according to treatment diet specifications. The premixed experimental ingredients were added to the basal diet (at the rate of 5g kg^{-1}) and mixed in a horizontal mixer for 3 minutes to ensure a homogenised mixture. The meal was then put through a 'cold' pelleter running at a maximum temperature of 40°C to produce 3mm diameter pellets.

Animal Husbandry

This experiment was performed using male (Ross 508) broiler chickens between 0 and 36 days of age. Day old chicks were obtained from a commercial hatchery (Mayfield Chicks, Lancashire) and randomly allocated to one of 32 identical experimental floor pens within eight environmentally controlled rooms. Each pen had a floor area of 1.83m^2 and housed 32 chickens, which complied with stocking density guidelines of 34kg liveweight m^2 at terminal weight. Pens were furnished with nipple drinkers and a suspended feeder, and shavings were used as litter. In order to increase the pathogenic challenge to the birds, an equal amount of 'old' litter was placed in each pen. The four experimental diets were randomised in 8 blocks of 4 pens with 8 treatment replicates. Thus there were 1024 birds in total, with 256 birds allocated to each treatment diet. Feed, presented in pellet form, and water were available *ad libitum*. The daily lighting pattern was maintained at 23 hours of artificial illumination throughout the experimental period. The temperature within the house was gradually reduced from 30°C at arrival until it reached 22°C, mirroring commercial conditions. Spot brooders were provided in each pen during the first week of the experiment.

Growth Performance Determination

Birds were weighed on a pen basis when allocated to the pens. The body weight change of each pen of birds was recorded at the end of each feeding period and expressed as g bird day^{-1}. Feed consumption for each pen was recorded over the two feeding periods and efficiency of conversion calculated. In the event of mortality, dead birds were removed following registration of date, body weight and pen number. The weights of dead birds were considered when calculating growth performance.

Dissections

On days 18 and 36 of the experiment, 2 birds per pen were randomly selected and killed by cervical dislocation. Liver, pancreas and caeca weights were recorded for each bird. Caeca were tied off and excised, immediately frozen in liquid nitrogen and stored at –80°C pending laboratory analysis. The digestive tract of each bird was examined for macroscopic coccidial lesions, and the presence/absence of *Eimeria tenella* and *Eimeria necatrix* lesions was recorded.

Litter Quality

Litter quality in each pen was scored on day 32 of the experiment using the scoring system seen in table 3.2.3. Water intake was measured on a pen basis over a 24 hour period on day 35 of the experiment. At 27 days of age, two birds from each pen were randomly selected and placed in the metabolism cages used in the previous experiments. After an adaptation period of two days, droppings were quantitatively collected over a four day period. Droppings were oven dried at 60°C, and dry matter calculated.

Table 3.2.3 Litter scoring criteria

Score	Interpretation
1	Free flowing/crumbly. No capping.
2	Very slight capping visible, but mostly friable. Any capping easily moved.
3	Access to friable litter partially reduced but approximately 50% of pen friable.
4	Most areas capped but friable litter still accessible in small areas.
5	Extensive capping with negligible access to friable litter.

Microbiological Analyses

Anaerobic microbiology was carried out on caecal samples taken from birds at 18 and 36 days of age. Analysis was conducted on 16 caecal samples per treatment group at each time point. Although it seems reasonable to assume that any effect of herbs on microflora populations would be seen in the proximal end of the small intestine where the majority of nutrient absorption occurs, bacterial counts are highly variable in this location due to the relatively short digesta transit time. Conversely, variations in bacterial counts are considerably smaller in the caeca (Hock *et al.*, 1997) where transit time is longer, and thus provides more stable anaerobic conditions. Although caecal bacterial counts are slightly higher than those in the ileum, they are comparable (Rubio *et al.*, 1998; Engberg *et al.*, 1999; Engberg *et al.*, 2000; Engberg *et al.*, 2002). It was necessary to freeze caecal samples prior to analysis for practical reasons. However, this was carried out as soon as possible *post mortem* and all samples received similar treatment. It is not clear if this freezing technique may have influenced the bacterial population composition. Bacterial groups have different cell wall characteristics, which may influence the physical strength of a cell to protect itself from bursting under freezing or thawing conditions (Clarke, 1977). Reports in the literature on the effects of

freezing on the viability of bacteria are scarce and confounding. Two groups at the same institute (Department of Animal Sciences, University of Illinois) investigating the effect of freezing on ruminal bacteria came to conflicting conclusions: Hsu and Fahey (1990) state that freezing ruminal fluid is not appropriate for obtaining truly representative bacterial samples, but Cecava *et al.* (1990) found that freezing of samples before isolation of mixed bacteria does not appear to affect composition. No reports on the effects of freezing on the viability of monogastric microflora could be found in the literature. All samples were treated in the same way so it was decided that any discrepancy as a result of freezing would be manifested across all treatments, therefore the results would still be comparable and valid.

Anaerobic microbiology was performed within a Mark 3 Anaerobic Workstation (Don Whitley Scientific, Yorkshire) using a gas mixture of carbon dioxide (10%), hydrogen (10%) and nitrogen (80%). All culture media and diluents were equilibrated in the workstation overnight prior to use in order to render them anaerobic.

Samples of approximately 1g of caecal material were weighed into sterile universal tubes, made up to ten times their mass by addition of sterile, anaerobic maximum recovery diluent (MRD) and thoroughly mixed. From these 1:10 dilutions, serial dilutions of up to 10^9 were made using sterile MRD. Within 2 hours of preparation, samples of 50µl were plated onto the appropriate culture media (Table 3.2.4) using a Model D Spiral Plater (Spiral Systems Inc., Ohio) and incubated at 37^0C (+/- 1^0C) under applicable atmospheric conditions (Table 3.2.4). Following incubation, viable bacteria colonies on each plate were enumerated using a ProtoCOL automated colony counter (Spiral Systems Inc., Ohio). After application of the appropriate dilution factor, bacterial counts in each sample were calculated as colony-forming units (cfu) per gram of caecal material. All analyses were carried out in triplicate.

Table 3.2.4 Cultures and conditions used for bacterial growth

Bacteria studied	Culture media	Incubation time	Atmosphere
Total aerobes	Luria-Bertani agar	24h +/- 6h	Aerobic
Total anaerobes	Fastidious anaerobe agar	48h +/- 6h	Anaerobic
Lactic acid bacteria	MRS agar	48h +/- 6h	Anaerobic
Escherichia coli	Eosin methylene blue agar	24h +/- 6h	Anaerobic
Bacteroides fragilis	Bacteroides bile esculin agar	24h +/- 6h	Anaerobic

All culture media supplied by Oxoid (UK) and prepared in accordance with maufacturer's instructions

Statistical Analyses

The effects of additives and feeding period on growth performance, relative organ weights, dry matter of droppings and water intake values were analysed statistically by analysis of variance using a factorial design (Genstat Release 5; Lawes Agricultural Trust, Rothamsted). Litter scores were compared using a Chi-squared test. All bacterial counts were transformed by conversion to $\log_{10}$, and log counts were analysed using a randomised block analysis of variance. Data from observations of coccidial lesions were pooled, and a Chi-squared test was used to compare the proportion of chickens within a treatment group showing lesions. All statements of significance are based on a probability of less than 0.05, although anything less than 0.1 has been indicated.

3.2.4 Results

In general, no differences in daily weight gain or feed conversion efficiency were observed in male broilers fed the different dietary treatments. From 0-18 days of age (starter period; table 3.2.5), the daily weight gains of broilers fed treatment diets not containing garlic were numerically higher than their garlic supplemented conspecifics, but this did not reach statistical significance (P=0.088). These birds also ate more feed (P=0.044), so FCE was not significantly different between the two groups. Mortality was low during the experimental period (3.7%), and was not affected by treatment diet (P>0.05).

Table 3.2.5 The growth performance of male broilers during the starter period (0 to 18 days) fed control and garlic supplemented diets

	Weight Gain	**Feed Intake**	**FCE**
	g bird day^{-1}		gain : feed
GARLIC SUPPLEMENTATION			
-	21.6	31.7	0.682
+	21.0	30.9	0.681
SEM	0.24	0.27	0.0041
P	0.085	**0.044**	NS

NS=P>0.1

Weight gains, feed intakes and feed conversion were not significantly different among birds fed garlic and non-garlic supplemented diets during the grower phase of the experiment (18-36d), as seen in table 3.2.6. In addition, no feeding period x garlic interactions were observed in any of the measured growth performance parameters.

Table 3.2.6 The growth performance of male broilers during the grower period (18 to 36 days) fed garlic supplemented diets with or without initial exposure to garlic

	Weight Gain	**Feed Intake**	**FCE**
	g bird day^{-1}		gain : feed
GARLIC SUPPLEMENTATION (starter: 0-18d)			
-	62.3	118.5	0.525
+	61.4	117.3	0.524
SEM	2.08	1.54	0.0160
P	NS	NS	NS
GARLIC SUPPLEMENTATION (grower: 18-36d)			
-	61.4	116.3	0.529
+	62.2	119.6	0.520
SEM	2.08	1.54	0.0160
P	NS	NS	NS
STARTER x GROWER GARLIC			
-/-	60.9	116.9	0.521
-/+	63.6	120.1	0.529
+/-	61.9	115.6	0.537
+/+	60.8	119.0	0.511
SEM	2.93	2.18	0.0226
P	NS	NS	NS

NS=*P*>0.1

Growth performance in general was below targets set by Ross Breeders for this strain of broiler. During the starter period (table 3.2.5), weight gains, feed intake and FCE were on average 36.3, 27.8 and 10.4% lower respectively. Similarly weight gains, feed intakes and FCE were 20.3, 12.8 and 8.6% respectively below targets during the grower period (table 3.2.6).

Again, there was no effect of garlic supplementation on growth performance throughout the experimental period (0-36 days; table 3.2.7), nor were there any interactions between feeding period and garlic supplementation.

Table 3.2.7 The growth performance of male broilers during the experimental period (0 to 36 days) fed garlic supplemented diets with or without initial exposure to garlic

	Weight Gain	Feed Intake	FCE
	g bird day^{-1}		gain : feed
GARLIC SUPPLEMENTATION (starter: 0-18d)			
-	41.9	75.0	0.557
+	41.2	74.1	0.556
SEM	1.09	0.810	0.0131
P	NS	NS	NS
GARLIC SUPPLEMENTATION (grower: 18-36d)			
-	41.4	73.9	0.560
+	41.8	75.2	0.553
SEM	1.09	0.81	0.0131
P	NS	NS	NS
STARTER x GROWER GARLIC			
-/-	41.2	74.3	0.554
-/+	42.6	75.8	0.561
+/-	41.5	73.5	0.566
+/+	40.9	74.7	0.546
SEM	1.55	1.15	0.0185
P	NS	NS	NS

NS=*P*>0.1

No treatment effects were observed on either proportional liver or proportional pancreas weights at any time during the experiment (Table 3.2.8), nor were there any interactions between garlic supplementation and feeding period. Proportional liver and pancreas weight ranges recorded were within ranges reported by other workers feeding herb supplemented diets (Jamroz *et al.*, 2003; Lee *et al.*, 2003a; Lee *et al.*, 2003b; Hernandez *et al.*, 2004; Jang *et al.*, 2004).

Table 3.2.8 Proportional liver and pancreas weights of 18 and 36 day old broilers fed garlic supplemented diets with or without initial exposure to garlic

	Liver weight	**Pancreas weight**
	(weights expressed as g kg^{-1} body weight)	
GARLIC SUPPLEMENTATION		
(starter: 0-18d)	*18 days*	*18 days*
-	25.5	3.6
+	27.2	3.5
SEM	0.69	0.13
P	NS	NS
GARLIC SUPPLEMENTATION		
(grower: 18-36d)	*36 days*	*36 days*
-	21.2	2.4
+	20.6	2.4
SEM	0.39	0.07
P	NS	NS
STARTER x GROWER GARLIC		
(0-36d)	*36 days*	*36 days*
-/-	20.6	2.4
-/+	20.6	2.4
+/-	21.9	2.4
+/+	20.5	2.3
SEM	0.056	0.09
P	NS	NS

NS=*P*>0.1

No treatment effects were observed in any of the litter quality parameters measured (table 3.2.9). Individual water intakes were within ranges quoted by other authors who fed herb supplemented diets to growing broilers (Lee *et al.*, 2003a; Lee *et al.*, 2003b; Lee *et al.*, 2004).

Table 3.2.9 Effect of treatment diet on litter quality parameters

Garlic starter/grower	Parameter measured			
	Litter score (day 32)		**Dry matter of droppings** (days 30-34)	**Water intake** (ml $bird^{-1}$ on day 35)
-/-	2.56		29.48	307.3
-/+	2.25		30.07	298.5
+/-	3.31		28.75	311.5
+/+	2.75		31.30	320.5
DF	3	SEM	1.321	13.77
χ^2	0.219	CV (%)	8.1	8.9
P	NS	*P*	NS	NS

χ^2 = Chi squared statistic; NS=*P*>0.1

At the end of the starter period, birds fed garlic diets had numerically lower prevalence of *E. necatrix* coccidial lesions than control fed birds (Table 3.2.10). Conversely presence of *E. tenella* lesions were numerically higher for garlic fed birds over the same period. However, these trends were not statistically proven (*P*>0.05). At the end of the experiment, the proportions of birds showing *E. necatrix* lesions were 0.406 and 0.376 for control and garlic fed birds respectively (*P*>0.05), and the proportions of birds showing *E. tenella* lesions were 0.376 and 0.344 for control and garlic fed birds respectively (*P*>0.05).

Table 3.2.10 Prevalence of coccidial lesions in birds at 18 and 36d of age

	Proportion of birds showing coccidial lesions				
	18d			36d	
Garlic	*Eimeria necatrix*	*Eimeria tenella*	Garlic starter/grower	*Eimeria necatrix*	*Eimeria tenella*
-	0.188	0.157	-/-	0.438	0.313
			-/+	0.438	0.438
+	0.157	0.188	+/-	0.375	0.313
			+/+	0.313	0.250
DF	1	1		3	3
χ^2	1	0.27		0.44	0.90
P	NS	NS		NS	NS

χ^2 = Chi squared statistic; NS=P>0.1

In view of the lack of growth performance differences between treatment diets, and owing to the extensive nature of the microbiological analysis, caecal bacterial populations were not investigated in this experiment.

3.2.5 Discussion

It was expected that feeding garlic powder would stimulate growth performance in broiler chickens. However, garlic powder did not influence weight gain, feed intake or efficiency of feed conversion during the experimental period. The dietary inclusion of garlic powder was considered to be sufficiently high on the basis of both *in vitro* antimicrobial reports (Naganawa *et al.*, 1996; Ankri and Mirelman, 1999; Harris *et al.*, 2001; Ross *et al.*, 2001; Yin *et al.*, 2002) and the previous experiment. The lack of effect on growth performance may relate to the composition of the basal diet. The diet contained highly-digestible ingredients which may have limited bacterial growth in the intestine. Other reports in the literature have

suggested that beneficial effects of herbal feed additives may be masked when highly digestible diets are fed (de Freitas *et al.*, 2001; Botsoglou *et al.*, 2002; Lee *et al.*, 2003a; Jang *et al.*, 2004).

The present study was designed to offer a situation typical of commercial growing conditions. It was thought that this would increase the likelihood of observing statistically improved growth performance. In the two previous experiments, growth effects were only seen when bird growth performance was below commercial targets. However, even though bird growth performance was much poorer than commercial targets set out by Ross Breeders, no effect of feeding garlic powder was seen. Reported growth response to garlic supplementation in broilers is highly variable, with either positive (Qureshi *et al.*, 1983a; Horton *et al.*, 1991b; Mottaghitalab, 2000; Tucker, 2002) or no (de Freitas *et al.*, 2001; Demir *et al.*, 2003; Cross *et al.*, 2004b) effects seen. Although *in vitro* testing of garlic rarely fails to show an antimicrobial response, it must be remembered that these tests are carried out using pure garlic in laboratory conditions. Herbal compounds are somewhat diluted in the digestive tract, and their synergism with other dietary components and the enteric environment may reduce their antimicrobial activity *in vivo*. Also, garlic is inherently volatile, and alliin, the component with the highest antimicrobial activity, is a highly unstable compound which rapidly breaks down into non-antimicrobial compounds (Block, 1985; Singh *et al.*, 1998; Ankri and Mirelman, 1999; Harris *et al.*, 2001). So it may be that the active principles in the garlic powder were rendered inactive as a result of this instability.

Litter quality can be of great importance in broiler production, particularly when wheat-based diets are fed. Wet litter is known to cause carcass downgrades at the slaughterhouse due to the increased incidence of breast blisters, skin burns, bruising and condemnations, and also to

increase ammonia production in broiler houses. It was thought that garlic supplementation may have a positive effect on litter, possibly as a result of a reduction in the amount of pathogenic bacteria, or through reduced water intake. However, no effects on litter quality were observed.

Similarly, prevelance of coccidial lesions (*E. tenella* and *E. necatrix*) was unaffected by garlic supplementation. Previous studies have demonstrated a reduction in lesion scores, incidence of bloody diarrhoea and faecal oocyst output after feeding herbs to chickens experimentally infected with *E. tenella* (Allen *et al.*, 1997; Youn and Noh, 2001; Giannenas *et al.*, 2003) but not *E. maxima* (Allen *et al.*, 1997) or *E. acervulina* (Allen *et al.*, 1997; Ibrir *et al.*, 2001; Ibrir *et al.*, 2002). However, no references of garlic being used as an anti-coccidial could be found in the literature. It is assumed, from the results in the present study, that the garlic powder used has no anti-coccidial properties.

The main objective of this study was to evaluate the potential of garlic powder as a growth enhancer in male broiler chickens reared in a situation typical of commercial conditions. Results indicate that dietary supplementation with the garlic powder used in the present study has no effect on growth performance, litter quality or coccidial load. Therefore, the investigation of garlic powder as a natural growth promotant will not be pursued in this series of experiments.

YARROW

3.2.6 Materials and Methods

With the exception of herbal premix used, the experimental design of this study was the same as the previous study, thus materials and methods are identical to those described previously (Section 3.2.3). The yarrow herbal premix contained 1800mg kg^{-1} dried yarrow plant w/w on a dextrose carrier, and was included at the rate of 50g kg^{-1}.

3.2.7 Results

Mortality rate during the experiment was 2.9%, and was unaffected by treatment diet (P>0.05).

No significant treatment differences in growth performance were observed during the initial feeding period (0-18 days) although feed intake tended (P=0.097) to be higher in the birds fed treatment diets not containing yarrow (table 3.2.11).

Table 3.2.11 The growth performance of male broilers during the starter period (0 to 18 days) fed control and yarrow supplemented diets

	Weight Gain	**Feed Intake**	**FCE**
	g bird day^{-1}		(gain : feed)
YARROW SUPPLEMENTATION			
-	22.6	34.1	0.662
+	22.0	33.0	0.668
SEM	0.44	0.45	0.0065
P	NS	0.097	NS

NS=P>0.1

Birds fed diets containing yarrow during the starter period had lower feed intakes ($P<0.05$) and a tendency for lower weight gains ($P=0.069$) during the grower period, although FCE was unaffected (table 3.2.12). Yarrow supplementation during the grower period had no effect on the measured growth performance during the grower period, although birds fed yarrow in the starter but not grower period (+/-) had lower daily weight gains ($P<0.05$) and feed intake ($P<0.05$) (table 3.2.12).

Table 3.2.12 The growth performance of male broilers during the grower period (18 to 36 days) fed yarrow supplemented diets with or without initial exposure to yarrow

	Weight Gain	**Feed Intake**	**FCE**
	g bird day^{-1}		(gain : feed)
YARROW SUPPLEMENTATION (starter: 0-18d)			
-	71.0	121.4	0.586
+	68.6	116.3	0.590
SEM	0.89	1.65	0.0067
P	0.069	**0.041**	NS
YARROW SUPPLEMENTATION (grower: 18-36d)			
-	69.0	116.9	0.591
+	70.7	120.8	0.585
SEM	0.89	1.65	0.0067
P	NS	NS	NS
STARTER x GROWER YARROW			
-/-	71.8	121.9	0.589
-/+	70.3	120.8	0.583
+/-	66.2	111.8	0.593
+/+	71.0	120.8	0.588
SEM	1.26	2.33	0.0095
P	**0.020**	**0.041**	NS

NS=$P>0.1$

Although no yarrow dietary treatment improved growth performance relative to the unsupplemented group throughout the experimental period, there was a dietary treatment x feeding period interaction on growth rate ($P=0.009$; Table 3.2.13) and a near interaction on

feed intake (P=0.052; Table 3.2.13). Birds fed yarrow supplemented diets during the starter period ate (P<0.05) and grew (P<0.05) less than their control fed conspecifics. However, during the grower period, yarrow supplementation tended to confer better growth rate (P=0.064) and higher feed intakes (P=0.097). Birds fed treatment diets containing yarrow during the grower period and throughout the experimental period had higher daily weight gains (P=0.009) and a tendency for higher feed intake (P=0.052) than those fed yarrow in the starter but not grower period.

Table 3.2.13 The growth performance of male broilers during the experimental period (0 to 36 days) fed yarrow supplemented diets with or without initial exposure to yarrow

	Weight Gain	**Feed Intake**	**FCE**
	g bird day^{-1}		(gain:feed)
YARROW SUPPLEMENTATION (starter: 0-18d)			
-	43.9	74.8	0.587
+	42.5	71.8	0.592
SEM	0.48	0.89	0.0046
P	**0.038**	**0.024**	NS
YARROW SUPPLEMENTATION (grower: 18-36d)			
-	42.5	72.2	0.589
+	43.9	74.4	0.590
SEM	0.48	0.89	0.0046
P	0.064	0.097	NS
STARTER x GROWER YARROW			
-/-	44.3	75.1	0.591
-/+	43.6	74.6	0.585
+/-	40.8	69.4	0.587
+/+	44.1	74.2	0.595
SEM	0.68	1.27	0.0065
P	**0.009**	0.052	NS

NS=P>0.1

There were no treatment effects on bird FCE throughout the experiment (Tables 3.2.11, 3.2.12 and 3.2.13), which was slightly below Ross performance objectives (grand mean of 0.59

compared with Ross targets of 0.62). Average bird weight was 4 days behind Ross performance objectives at 36 days (grand mean 1689g; Ross targets 1688g and 2041g at 32 and 36 days respectively).

No treatment effects were observed on either proportional liver or proportional pancreas weights at any time during the experiment (Table 3.2.14), nor were there any interactions between yarrow supplementation and feeding period. Proportional liver and pancreas weight ranges recorded were within ranges reported by other workers feeding herb supplemented diets (Jamroz *et al.*, 2003; Lee *et al.*, 2003a; Lee *et al.*, 2003b; Hernandez *et al.*, 2004; Jang *et al.*, 2004).

Table 3.2.14 Proportional liver and pancreas weights of 18 and 36 day old broilers fed yarrow supplemented diets with or without initial exposure to yarrow

	Liver weight	**Pancreas weight**
	(weights expressed as g kg^{-1} body weight)	
INITIAL YARROW		
(starter: 0-18d)	*18 days*	*18 days*
-	28.9	3.9
+	27.6	3.8
SEM	0.73	0.13
P	NS	NS
CURRENT YARROW		
(grower: 18-36d)	*36 days*	*36 days*
-	24.4	2.3
+	24.3	2.4
SEM	0.53	0.08
P	NS	NS
INITIAL x CURRENT YARROW		
(starter x grower: 0-36d)	*36 days*	*36 days*
-/-	24.5	2.2
-/+	25.9	2.4
+/-	24.3	2.4
+/+	22.8	2.4
SEM	0.75	0.11
P	NS	NS

NS=*P*>0.1

Litter score data were normally distributed, and so were analysed using ANOVA. No treatment effects were observed in any of the litter quality parameters measured (Table 3.2.15).

Table 3.2.15 Effect of treatment diet on litter quality parameters

			Parameter measured	
Yarrow starter/grower	**Litter score** (day 32)		**Dry matter of droppings** (days 30-34)	**Water intake** (ml bird^{-1} on day 35)
-/-	2.56		29.27	307.3
-/+	3.06		28.90	298.5
+/-	2.44		28.63	311.5
+/+	2.88		29.29	320.5
DF	3	SEM	1.108	9.74
χ^2	0.089	CV (%)	10.8	8.9
P	NS	*P*	NS	NS

χ^2 = Chi squared statistic; NS=*P*>0.1

Prevalence of coccidial lesions were not affected by treatment diet (*P*>0.05) at either 18 or 36 days of age (Table 3.2.16). At the end of the starter period, a higher proportion of birds showed *E. tenella* lesions, and yarrow fed birds had higher incidence of these lesions than controls (*P*>0.05). At the end of the experiment, the proportions of birds showing *E. necatrix* lesions were 0.344 and 0.313 for control and yarrow fed birds respectively (*P*>0.05), and the proportions of birds showing *E. tenella* lesions were 0.469 and 0.375 for control and yarrow fed birds respectively (*P*>0.05).

Table 3.2.16 Prevalence of coccidial lesions in birds at 18 and 36d of age

	Proportion of birds showing coccidial lesions				
	18d			36d	
Yarrow	*Eimeria necatrix*	*Eimeria tenella*	Yarrow starter/grower	*Eimeria necatrix*	*Eimeria tenella*
-	0.251	0.094	-/-	0.313	0.375
			-/+	0.313	0.438
+	0.251	0.157	+/-	0.375	0.563
			+/+	0.313	0.313
DF	1	1		3	3
χ^2	1.3	3.4		2.1	2.2
P	NS	NS		NS	NS

χ^2 = Chi squared statistic; NS=P>0.1

Addition of yarrow did not affect caecal bacterial populations (P>0.05) at either 18 or 36 days of age, nor were there any interactions between yarrow supplementation and feeding period (Table 3.2.17). Caecal bacterial counts were within the ranges quoted by authors feeding yarrow (Cross *et al.*, 2001) and traditional AGP (Engberg *et al.*, 2002).

Lactic acid bacteria produce lactic acid which reduces the pH of the intestinal contents below the optimum range of pathogenic bacteria such as *E. coli.* This reduction in gut pH is consistent with the proposed modes of action for organic acids and some probiotics. Work by Fuller (1973) has demonstrated that *E. coli* numbers in the crop of adult chickens increase when lactic acid bacteria are eliminated through dietary penicillin, and thus *Lactobacilli* are directly involved in preventing the unrestricted growth of *E. coli in vivo.* However, this inhibitory effect is dependant on sufficient numbers of *Lactobacilli* being present (Fuller, 1977), so proportions of lactic acid bacteria to *E. coli* provide a good indication of gut health. However, yarrow supplementation did not affect counts of lactic acid bacteria, *E. coli* or the lactic acid:*E. coli* ratio (P>0.05) at any time during the experiment.

Table 3.2.17 Bacteria counts from caeca taken from broilers fed control and yarrow supplemented diets with and without prior experience of yarrow

	18d				36d			
	-	+	SEM	*P*	-	+	SEM	*P*
Total bacteria	10.27	10.04	0.084	NS	10.63	10.74	0.112	NS
Total anaerobes	9.83	9.67	0.238	NS	9.91	9.94	0.093	NS
Coliforms	7.65	7.30	0.595	NS	7.48	7.16	0.384	NS
Lactobacillus	9.69	9.53	0.201	NS	9.09	9.24	0.146	NS
Bacteroides fragilis	9.75	9.26	0.242	NS	9.64	9.53	0.127	NS
Ratio of *Lactobacillus* to coliforms	1.27	1.32	0.109	NS	1.28	1.34	0.073	NS

	Yarrow starter/grower				SEM	CV (%)	*P*
	-/-	-/+	+/-	+/+			
Total bacteria	10.71	10.67	10.55	10.82	0.158	4.2	NS
Total anaerobes	9.98	9.90	9.84	9.98	0.131	3.7	NS
Coliforms	6.90	7.39	8.06	6.93	0.543	21.0	NS
Lactobacillus	9.24	9.23	8.94	9.25	0.206	6.4	NS
Bacteroides fragilis	9.761	9.530	9.518	9.539	0.1795	5.3	NS
Ratio of *Lactobacillus* to coliforms	1.420	1.294	1.137	1.378	0.1032	22.3	NS

Values are expressed as $\log_{10}$ colony forming units per gram of caecal material; NS=$P>0.1$

3.2.8 Discussion

This experiment demonstrated that yarrow supplementation of broiler chicken diets tends to mediate a positive growth response, but only in the latter part of the growth period (18-36 days). This is in agreement with the previous experiment where caged birds responded positively to yarrow supplementation between 17 and 27 days of age, but not between 7 and 17 days of age. Similarly Cross *et al.* (2002) reported that feeding yarrow had no effect on 7-21 day old broilers, but enhanced growth performance from 21-28 days.

AGP are thought to improve growth performance of non-ruminants through alteration of the gut microflora in a beneficial manner by selectively binding to pathogenic microorganisms in the gut. With less pathogenic species inhabiting the gut, beneficial microorganisms, such as *Lactobacilli* are able to proliferate and further suppress the growth of harmful species (Vandevoorde *et al.*, 1991; Hinton *et al.*, 1992; Pascauli *et al.*, 1999) thus improving feed digestibility, nutrient utilisation and subsequent growth performance (Schneitz *et al.*, 1998). Yarrow supplementation may also result in a change in gut microbial populations, which has been demonstrated previously in broilers fed other herbal products (Williams and Losa, 2001; Tucker, 2002; Jamroz *et al.*, 2003). Results from the present experiment indicate that yarrow supplementation does have an effect on growth performance, but the caecal microbiology carried out has discredited the hypothesis that this response was mediated through a beneficial effect on gut microflora, which agrees with the findings of Cross *et al.* (2001).

As with the garlic study, no effects on litter quality or coccidial load were observed in this experiment. Engberg *et al.* (1996) suggested that the improvement in litter quality seen when AGP were fed was a result of a reduction in pathogenic bacteria within the tract. No effect on

microflora was observed in the present study, which may explain the lack of effect on litter quality. Again, no specific reference to yarrow having anticoccidial effects could be found in the literature, although it is reported to be active against helminths (Cowan, 1999). It must be assumed that the yarrow used in the present study does not have any anticoccidial properties.

Concentrations of gastrointestinal bacteria were not significantly affected ($P>0.05$) by dietary treatment at either 18 or 36 days of age, nor were there any interactions between yarrow supplementation and feeding period. Similar work carried out in this area (Cross *et al.*, 2001) also failed to demonstrate a link between yarrow supplementation and moderation of gut flora. Samarasinghe *et al.* (2003) observed reduced duodenal coliforms ($P<0.05$) relative to controls when turmeric was fed (1g kg^{-1}), with a concomitant increase in performance. Similarly, Jamroz *et al.* (2003) reported a reduction in faecal *E. coli* numbers ($P<0.05$) in 40 day old broilers fed an essential oil supplement containing oregano, capsicum and cinnamon extracts. However, Jang and workers (2004) found no effect on ileal *Lactobacillus* or *E. coli* numbers at 35 days when feeding the same premix, even though a positive growth response was noted.

It is not clear why yarrow supplementation had no effect on gut microbial populations. Replication of microbiological analysis was considered to be sufficient to detect a response ($n=16$). Dorman and Deans (2000) suggested that the active terpenoid phytochemicals in the whole plant might be trapped within secretory gland structures, which may favour the antimicrobial activity of plant oils rather than plant herbs. The drying process may have affected the constituent terpenoid concentration and phytochemicals with less antibacterial activity may have prevailed within the plant due to their more stable nature. Cross and workers (2002) investigated the differences in growth response when feeding yarrow herb and oil fractions and demonstrated large discrepancies in growth performance between the two

derivatives. Herb fed birds attained higher weight gains (P=0.001) and improved feed conversion efficiency (P<0.05) in comparison with their oil supplemented conspecifics implying that the active constituents do vary between the herb and oil fractions of yarrow, and indicating that the herb derivative has a more positive growth effect than the oil derivative.

This experiment has demonstrated that yarrow supplementation of broiler chicken diets appears to mediate a growth response, but only in the latter part of the growth period (18-36 days). This is consistent with the findings of experiment 2 (sections 3.1.6-3.1.8) where caged birds responded positively to yarrow supplementation between 17 and 27 days of age, but not between 7 and 17 days of age. Similarly Cross *et al.* (2002) reported that feeding yarrow had no effect on the growth performance of 7-21 day old broilers, but enhanced growth performance from 21-28 days. It was postulated that yarrow mediates its positive effects through beneficial moderation of the gut microflora. However, no effects were observed. It may be that the antimicrobial activity seen *in vitro* is reduced or not seen *in vivo*. This thesis seems plausible as another group (Cross *et al.*, 2001) also failed to demonstrate a link between yarrow supplementation and moderation of bacterial flora. Yarrow composition is highly variable (table 2.8), and little is known about which components are responsible for the different actions seen *in vitro* and *in vivo*. It is known however that 1,8-cineole is the component with the strongest antibacterial properties (Candan *et al.*, 2003). Interestingly, the yarrow sample used in the *in vitro* antimicrobial studies carried out by Candan *et al.* (2003) had much higher levels of 1,8-cineole than both of the yarrow samples used by Cross *et al.* (2001) (24.6 *vs.* 0 and 1.88 mg g^{-1} for the samples used by Candan and Cross respectively). It is possible that the yarrow used in this feeding study was also low in 1,8-cineole, which may explain the lack of effect on the gut flora.

3.3 ARE THE EFFECTS OF YARROW SUPPLEMENTATION MORE PRONOUNCED WHEN LESS NUTRIENT DENSE DIETS ARE FED?

3.3.1 Introduction

Published work on herbal growth promotion often discusses the possibility that failure to detect beneficial growth responses in feeding experiments may be attributed to feeding highly digestible basal diets (de Freitas *et al.*, 2001; Botsoglou *et al.*, 2002, Lee *et al.*, 2003, Jang *et al.*, 2004). The effects of dietary fibre on nutrient digestibility in monogastrics have been well documented (Annison, 1990; Choct and Annison, 1990; Gdala, 1998; Pluske *et al.*, 2001). Generally, these authors report that fibre increases intestinal transit time, delays gastric emptying, delays glucose absorption, increases pancreatic secretions and assists faecal bulking. Dietary fibre often enhances dry matter flow and endogenous losses, leading to a decrease in ileal and faecal digestibility of energy and nutrients including starch, protein and lipids. As a result of these negative influences on digestion, dietary fibre is considered to be anti-nutritional (Montagne *et al.*, 2003).

Anecdotal evidence suggests that the 'bitter' flavours found in yarrow may stimulate digestive enzyme secretion and nutrient digestibility (Moerman, 1977; Hoffman, 1988; Chandler, 1992a; Leung and Foster, 1996; McCartney, 2002). Herbal supplementation has already been demonstrated to affect pancreatic enzyme secretion in rats (Platel and Srinivasan, 1996; Platel and Srinivasan, 2000) and digestive enzyme activity in growing broiler chickens (Lee, 2003a; Jang, *et al.*, 2004). In addition, Engberg *et al.* (2000) demonstrated that dietary addition of an AGP (zinc bacitracin) increased both pancreatic lipase and amylase activity ($P<0.05$), with a concomitant positive ($P<0.05$) growth response.

The present feeding experiment was therefore devised to describe the effects of dietary yarrow and its interaction with diet composition on growth performance, pancreatic enzyme activities and nutrient digestibility in male broiler chickens.

3.3.2 Specific Objectives

The hypothesis to be tested was that the positive effect of yarrow supplementation is more pronounced when high fibre basal diets are fed, and that its mode of action is linked to an increase in nutrient digestibility through increased activity of pancreatic enzymes. Thus, the specific objectives of the experiment were to investigate:

i. growth performance of caged male broilers fed one of two diets with and without yarrow supplementation over two feeding periods in a 2x2x2 factorial design
ii. pancreatic enzyme activity and nutrient digestibility

3.3.3. Material and Methods

Feed additives

The herbal premixes were provided by Braes Feed Ingredients (Chester, UK) and were from the same batch as those used in previous experiments. Prior to GC analysis, the herb material was distilled in order to produce an essential oil. Briefly, 100 grams of herb material was placed into a flask to which was added sufficient deionised water to cover the herb material. This was then distilled using the British Pharmacopoeia Clevenger distillation apparatus. The main components identified by GC analysis of the essential oil are shown in table 3.3.1.

Table 3.3.1 Analysis of yarrow essential oil

Main Components	% Composition
α-pinene	1.3
1,8-cineole	2.9
γ-terpinene	0.5
ρ-cymene	1.1
Camphor	14.1
Linalol	0.6
Caryophyllene	1.6
Borneol	22.1
α-terpineol	4.4
Cadinol	4.7
Cadinene	6.7
Germacrene	1.6
Total number of peaks	**55**
Total % of oil identified	**61.6**

Ration formulation

Broilers were fed one of four treatment diets during two feeding periods in a 2x2x2 factorial design, thus there were eight treatment diets in total (Figure 3.3.1). Feeding periods were starter (0-18 days) and grower (18-36 days). Control basal diets were wheat/soya bean meal based and complied with published recommendations for growing broiler chickens (NRC, 1994); low nutrient density diets were formulated to be approximately 100g kg^{-1} less nutrient dense than control diets and included high fibre, less digestible components. Each diet was supplemented with 50g kg^{-1} of either the yarrow product (1800mg kg^{-1} dried yarrow plant w/w on a dextrose carrier) or dextrose (control). No additional antimicrobials, anticoccidials or enzymes were used. Feed, presented in pellet form, and water were available *ad libitum.*

Ingredient composition and calculated analysis of the basal starter and grower diets are shown in tables 3.3.2 and 3.3.3 respectively.

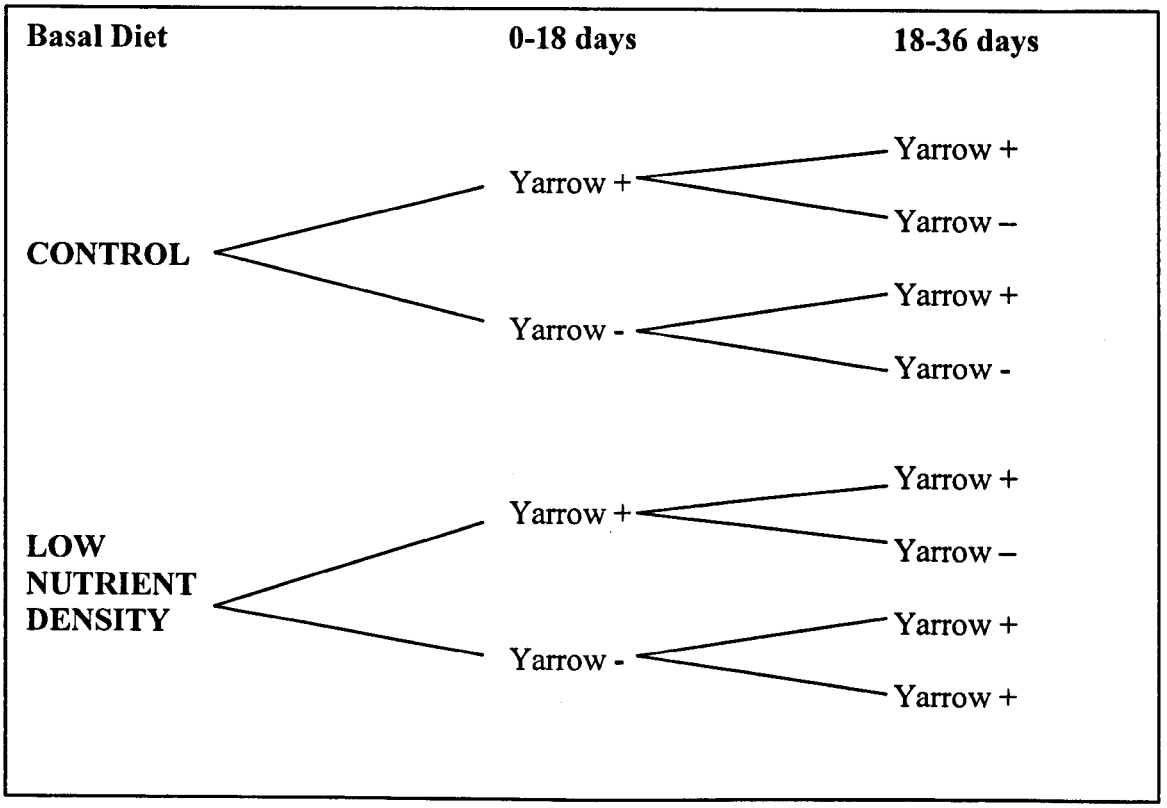

Figure 3.3.1 Dietary treatments

Table 3.3.2 Ingredient composition and calculated analysis of starter diets

Feedingstuff	Inclusion rate (kg tonne^{-1})	
	Adequate Diet	**Low nutrient density Diet**
Wheat	615	500
Hipro soya bean meal	133	40
Full fat soya bean meal	133	40
Wheat Feed	-	150
Sunflower Seed Meal	-	150
Fishmeal	50	50
Soya oil	25	25
Lysine HCl	1.5	3.3
Methionine	2.5	1.7
Limestone	7	7
Dicalcium phosphate	5	5
Salt	3	3
Vitamin and trace mineral premix [1]	20	20
Herbal product/dextrose control	5	5
Total	**1000**	**1000**
Calculated analysis		
Nutrient	**Concentration (per kg dry matter [2])**	
Metabolisable Energy	13.0 MJ	11.7 MJ
Crude fat	42.6g	44.8g
Crude protein	216g	193g
Crude fibre	30g	68g
Lysine	12.9g	11.4g
Methionine and cystine	9.3g	8.3g
Calcium	10.8g	10.9g
Phosphorus	5.8g	6.6g
Sodium	1.9g	1.9g

1 The vitamin and trace mineral premix contained vitamins and trace elements to meet the requirements specified by NRC (1994). The major components were: phosphorus, 95g/kg; calcium, 219g/kg; sodium, 30g/kg; copper sulphate, 0.5g/kg; selenium, 10mg/kg; retinol acetate, 0.275g/kg; cholecalciferol, 625mg/kg; alpha tocopherol, 2.273g/kg. The product was sourced from Ian Hollows Feed Supplements, Whitchurch, Shropshire.

2 Calculated dry matter 873 and 875g kg^{-1} for adequate and low nutrient density diets respectively

Table 3.3.3 Ingredient composition and calculated analysis of grower diets

Feedingstuff	**Inclusion rate (kg tonne^{-1})**	
	Adequate Diet	**Low nutrient density Diet**
Wheat	600	510
Hipro soya bean meal	140	93
Full fat soya bean meal	190	66
Wheat Feed	-	150
Sunflower Seed Meal	-	110
Soya oil	25	25
Lysine HCl	1.5	3.3
Methionine	2.5	2.4
Limestone	7	6.3
Dicalcium phosphate	5	5
Salt	4	4
Vitamin and trace mineral premix [1]	20	20
Herbal product/dextrose control	5	5
Total	**1000**	**1000**
Calculated analysis		
Nutrient	**Concentration (per kg dry matter [2])**	
Metabolisable Energy	13.1 MJ	11.9 MJ
Crude fat	38.9g	41.0g
Crude protein	205g	185g
Crude fibre	33g	61g
Lysine	11.9g	10.67g
Methionine and cystine	8.7g	8.4g
Calcium	9.0g	8.8g
Phosphorus	4.9g	5.5g
Sodium	1.9g	1.9g

[1] The vitamin and trace mineral premix contained vitamins and trace elements to meet the requirements specified by NRC (1994). The major components were: phosphorus, 95g/kg; calcium, 219g/kg; sodium, 30g/kg; copper sulphate, 0.5g/kg; selenium, 10mg/kg; retinol acetate, 0.275g/kg; cholecalciferol, 625mg/kg; alpha tocopherol, 2.273g/kg. The product was sourced from Ian Hollows Feed Supplements, Whitchurch, Shropshire.

[2] Calculated dry matter 871 and 873g kg^{-1} for adequate and low nutrient density diets respectively

Diet Manufacture

The feed was manufactured at Harper Adams University College. Experimental diets were mixed in 25kg batches according to treatment diet specifications. Appropriate concentrations of either yarrow product or dextrose were added to the diet and mixed in a horizontal mixer for 3 minutes to ensure a homogenised mixture. The meal was then put through a 'cold' pelleter running at a maximum temperature of 40°C to produce 3mm diameter pellets.

Animal Husbandry

The experiment was performed using male (Ross 508) broiler chickens between 0 and 36 days of age. Birds were weighed and randomly assigned to cages in groups of four, with each cage serving as a treatment replicate. The cages were housed in an environmentally controlled metabolism room. There were 48 cages in total, thus six replicates per treatment diet. Cages were arranged in two tiers. Birds were given free access to feed and water at all times. Feed was presented in pellet form. The temperature was maintained initially at 32^0C and reduced by 1^0C daily until it reached 22^0C. A one hour period of darkness was provided daily.

Growth Performance Determination

All growth performance data were collected on a cage basis. Birds were weighed when placed in the cages. Bird weight and feed consumption were determined on days 18 and 36 of the experiment. In the event of a mortality, dead birds were removed following registration of date and body weight. Body weights of dead animals were considered when calculating feed conversion efficiency.

Dissections

On days 18 and 36 of the experiment, 2 birds per cage were killed by cervical dislocation, individually weighed and dissected. At 18 days of age the two birds were selected randomly, and at 36 days the remaining birds were sampled. Food was withdrawn from birds 3 hours prior to digesta collection and replaced for 2 hours prior to digesta collection (Short *et al.*, 1999). This ensured that birds had all eaten prior to sampling, and that food withdrawal did not affect the steady state of the gastro-intestinal tract and that sufficient digesta samples were obtained. Digesta was obtained from the proximal part of the small intestine (duodenum plus jejunum) defined as the segment between the pylorus to Meckel's diverticulum (Knarreborg *et al.*, 2003, Lee *et al.*, 2003a). After rapid removal of this section digesta were gently squeezed, using digital pressure, into a collection vessel. Digesta samples from the two birds in each cage were pooled and quickly frozen at –20°C pending laboratory analyses. Liver and pancreas weights were recorded.

Digestive Enzyme Activity

Intestinal duodenum plus jejenum contents were individually homogenised in 4 volumes of ice-cold distilled water and centrifuged at 6500 x *g* for 15 minutes at 4^0C (Lee *et al.*, 2003a). Aliquots of the supernatant were removed for determination of amylase (EC 3.2.1.1), lipase (EC 3.1.1.3), trypsin (EC 3.4.21.4) and chymotrypsin (EC 3.4.21.1) activity. Duplicate analyses were carried out on two samples from each pooled sample of digesta collected.

Amylase

Amylase activity was measured using a Synermed Amylase test kit VI400 (Synermed Europe Limited, West Sussex) on a Cobas Mira Plus blood analyser (ABX Diagnostics, Montpellier, France) using a direct method with the patented substrate 2-chloro-*p*-nitrophenyl-α-D-

maltotrioside (CNPG3) which reacts directly with α-amylase. The substrate (CNPG3) is hydrolysed by α-amylase to release 2-chloro-*p*-nitrophenol (CNP) which is monitored spectrophotometrically at 405 nm. The hydrolysis products also include 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG2), maltotriose and glucose. The rate of formation of the CNP is directly proportional to the amount of amylase present in the sample.

Lipase

Lipase activity was measured using a Randox Lipase test kit (Catalogue number LI 194) on a Cobas Mira Plus blood analyser (ABX Diagnostics, Montpellier, France) using a turbidimetric method (Ziegenhorn *et al.*, 1979). The principle of this assay is that triolein and water form monoglyceride and oleic acid in the presence of lipase. The decrease in turbidity of this reaction was measured at 340 nm.

Trypsin

Trypsin activity was measured according to the method of Hummel (1959) using *p*-toluenesulphonyl-L-arginine methyl ester (TAME; Sigma Chemical Company) as a substrate. The rate of hydrolysis of TAME was measured by the increase in absorbency at 247nm. One unit of trypsin activity is defined as 1μmole of TAME hydrolysed per minute at 37°C and pH 8.1 in the presence of 10 mmoles $CaCl_2$ l^{-1}.

Chymotrypsin

Chymotrypsin activity was also measured according to the method of Hummel (1959). The rate of hydrolysis of benzoyl-L-tyrosine ethyl ester (BTEE; Sigma Chemical Company) was measured by the change in absorbency at 256nm. One unit of chymotrypsin activity is defined as 1μmole of BTEE hydrolysed per minute at 37^0C and pH 7.8.

AME Determination

Apparent Metabolisable Energy (AME) was determined using a total collection method (Sibbald, 1989) over four days: days 24-27 of the experiment. Representative homogenised samples of droppings (500g) were oven-dried at 60^0C for 48 hours, and dry matter content calculated. The gross energy of each dried droppings sample and the experimental diets were determined using a Parr 1261 adiabatic bomb calorimeter (Parr Instrument Company, USA). The AME of the eight diets was calculated by deducting the amount of energy contained in the collected droppings from the energy intakes of the birds over the four-day period.

Amino Acid Digestibility

Concentrations of amino acids were determined for the treatment diets and the droppings samples collected during the AME study. Droppings were freeze-dried (Model B, series 6/13; Girovac Limited, Hertfordshire) prior to analysis and ground to pass through a 0.25mm screen. Amino acid concentrations were determined according to standard methods (AOAC, 2000). Samples were hydrolysed in 6M hydrochloric acid for 18 h at 110^0C under nitrogen (or lithium hydroxide for tryptophan determination), and oxidised using performic acid to avoid cystine and methionine loss. Samples were then dried in a rotary evaporator and dissolved in 0.2M sodium buffer (2ml) and the pH adjusted to 2 with 10M sodium hydroxide. Aliquots of these samples were assayed on a cation exchange column, with nor-leucine as the internal standard. The amino acids were eluted using sodium citrate buffers and the eluted amino acids were detected by a ninhydrin colour reaction at 570nm for all amino acids except proline which was detected at 440nm. Apparent digestibility of amino acids was calculated according to Raharjo and Farrell (1984):

Apparent AA digestibility = (Feed AA - Droppings AA)/Feed AA

Statistical Analyses

The effects of yarrow supplementation and basal diet on growth performance, dissection data, AME values, dry matter content of droppings, digestive enzyme activity and amino acid digestibility were examined statistically by analysis of variance using a factorial design (Genstat Release 5: Lawes Agricultural Trust, Rothamsted). Data obtained from the dissections and digestive enzyme activity assays were pooled for the two birds within each replicate cage before statistical analysis. All statements of significance are based on a probability of less than 0.05, although anything less than 0.1 has been indicated.

3.3.4 Results

Mortality was low throughout the experimental period (3.1%) with no appreciable differences being observed between the treatment groups. At 36 days of age, bird body weight varied from 1633 to 2158g, with a grand mean of 2054g, comparing favourably with Ross commercial broiler targets of 2041g. As expected, birds fed the low nutrient density treatment diets showed lower DLWG than control fed birds throughout the experimental period (0-18d, $P<0.001$; 18-36d, $P=0.081$; 0-36d, $P=0.020$) with a concomitant effect on FCE ($P<0.001$). This is consistent with the results of previously reported experiments involving diet dilution (Arija *et al.*, 1998). Birds fed the low nutrient density diets were, on average, 157g lighter ($P=0.004$) than their control fed counterparts at 36 days of age.

Birds performed well during the starter period (table 3.3.4), achieving a grand mean daily weight comparable with Ross commercial targets over this period (31.7 *vs.* 32.5g bird day^{-1} for experimental birds and Ross targets respectively). However, feed intake was slightly higher which led to marginally poorer feed conversion efficiency. Yarrow supplementation did not

improve any growth performance variables when fed as part of either control or low nutrient density diets during the starter period (P>0.05, table 3.3.4).

Table 3.3.4 The growth performance of male broilers during the starter period (0-18d) fed control and low nutrient density basal diets with and without yarrow supplementation

Diet	Performance Variables	Yarrow		Mean Diet Dilution Effects
		-	+	
Control	DLWG (g day^{-1})	33.9	32.8	33.4
	DFI (g day^{-1})	50.1	48.8	49.5
	FCE	0.678	0.673	0.676
Low nutrient density	DLWG (g day^{-1})	29.9	30.1	30.0
	DFI (g day^{-1})	50.8	50.8	50.3
	FCE	0.593	0.602	0.597
	Mean Yarrow Effects			
	DLWG (g day^{-1})	31.9	31.5	
	DFI (g day^{-1})	50.3	49.4	
	FCE	0.636	0.637	

	Statistical significance and SEM of treatment means					
	Diet		**Yarrow**		**Diet x Yarrow Interaction**	
	P	SEM	*P*	SEM	*P*	SEM
DLWG	**<0.001**	0.30	NS	0.30	NS	0.43
DFI	NS	0.50	NS	0.50	NS	0.70
FCE	**<0.001**	0.0055	NS	0.0055	NS	0.0074

NS=P>0.1

Again, performance was commensurate with Ross targets during the grower phase of the experiment. As expected, birds fed the low nutrient density diets had lower daily gains than control fed birds, although this was not statistically proven (P=0.080). It was thought that birds fed the low nutrient dense diets would eat more than their control fed conspecifics as a result of the diet dilution, but this was not apparent (table 3.3.5). There was a trend (P=0.087) for an interaction between diet and yarrow supplementation: yarrow supplementation reduced

daily feed intake of birds fed control diets by 6.3% but increased intake of birds fed the low nutrient density diets by 5.7%.

Table 3.3.5 The growth performance of male broilers during the grower period (18-36d) fed control and low nutrient density basal diets with and without yarrow supplementation during the starter and grower phases

Diet	Performance Variables	Yarrow		Mean Diet Dilution Effects
		-	+	
Control	DLWG (g day^{-1})	82.1	76.7	79.4
	DFI (g day^{-1})	152.6	143.1	147.8
	FCE	0.537	0.539	0.538
Low nutrient density	DLWG (g day^{-1})	72.7	75.3	74.0
	DFI (g day^{-1})	149.8	158.3	154.0
	FCE	0.484	0.477	0.480
	Mean Yarrow Effects			
	DLWG (g day^{-1})	77.4	76.0	
	DFI (g day^{-1})	151.2	150.7	
	FCE	0.512	0.508	

	Statistical significance and SEM of treatment means					
	Diet		**Yarrow**		**Diet x Yarrow Interaction**	
	P	SEM	*P*	SEM	*P*	SEM
DLWG	0.081	0.30	NS	0.30	NS	0.43
DFI	NS	0.36	NS	0.36	0.087	0.51
FCE	**<0.001**	0.0087	NS	0.0087	NS	0.0123

NS=*P*>0.1

During the experimental period (0-36 days, table 3.3.6) birds fed the low nutrient density diets had 7.8% lower weight gains than their control fed counterparts ($P<0.05$) and as a result FCE was nearly 6 points lower ($P<0.001$). Birds fed non-supplemented control diets throughout the experiment ate more ($P<0.05$) than birds fed the other treatment diets, but also had a tendency for higher daily weight gains ($P=0.093$) so FCE was unaffected. There were no interactions between diet, yarrow supplementation and feeding period. However, birds fed yarrow supplemented diets during the starter period ate less feed than non-supplemented birds overall (90.2 *vs.* 86.6g bird day^{-1} for non-yarrow and yarrow supplemented birds respectively,

$P<0.05$) but attained similar final body weights at 36 days of age ($P>0.05$; table 3.3.8) indicating an improvement in efficiency. There was also a trend ($P=0.069$) for an interaction between diet and yarrow supplementation during the starter period on FCE, where yarrow supplementation during the starter period improved overall (0-36d) FCE by 4 points in control fed birds, but reduced it by 2 points in birds fed the low nutrient density diets.

Table 3.3.6 The growth performance of male broilers during the experimental period (0-36d) fed control and low nutrient density basal diets with and without yarrow supplementation during the starter and grower phases

Diet	**Performance Variables**	**Yarrow** *starter/grower*				**Mean Diet Dilution Effects**
		-/-	-/+	+/-	+/+	
Control	DLWG (g day^{-1})	54.2	48.8	51.5	51.3	51.5
	DFI (g day^{-1})	92.7	87.2	83.6	85.4	87.2
	FCE	0.584	0.560	0.619	0.601	0.591
Low nutrient density	DLWG (g day^{-1})	50.9	47.2	45.5	47.2	47.7
	DFI (g day^{-1})	92.7	88.1	86.9	90.4	89.5
	FCE	0.550	0.535	0.524	0.520	0.532
	Mean Yarrow Effects					
	DLWG (g day^{-1})	52.6	47.9	48.5	49.2	
	DFI (g day^{-1})	92.7	87.7	85.3	87.9	
	FCE	0.567	0.547	0.571	0.560	

	Statistical significance and SEM of treatment means					
	Diet		**Yarrow x feeding period interaction**		**Diet x Y x feeding period Interaction**	
	P	SEM	*P*	SEM	*P*	SEM
DLWG	**0.020**	1.54	0.093	2.18	NS	3.08
DFI	NS	1.57	**0.020**	2.23	NS	3.15
FCE	**<0.001**	0.0155	NS	0.0219	NS	0.0310

NS=$P>0.1$

Table 3.3.7 Proportional liver and pancreas weights of male broilers taken at the end of the starter period (18d) fed control and low nutrient density basal diets with and without yarrow supplementation

Diet	Performance Variables	Yarrow		Mean Diet Dilution Effects
		-	+	
Control	bird weight	663.2	651.0	657.1
	liver weight	32.32	34.55	33.44
	pancreas weight	3.38	3.31	3.35
Low nutrient density	bird weight	600.0	597.7	599.0
	liver weight	34.64	36.22	35.43
	pancreas weight	3.34	3.21	3.27
	Mean Yarrow Effects	-	+	
	bird weight	631.6	624.4	
	liver weight	33.48	35.39	
	pancreas weight	3.31	3.26	

	Statistical significance and SEM of treatment means					
	Diet		**Yarrow**		**D x Y Interaction**	
	P	SEM	*P*	SEM	*P*	SEM
bird weight	**<0.001**	8.68	NS	8.68	NS	12.27
liver weight	**0.011**	0.542	**0.015**	0.542	NS	0.766
pancreas weight	NS	0.073	NS	0.073	NS	0.103

NS=P>0.1

Pancreas weights were not affected by dietary yarrow (P>0.05) at either 18 (table 3.3.7) or 36 days of age (table 3.3.8). However, birds fed yarrow treatment diets up to 18 days of age tended to have heavier pancreas weights at 36 days (2.148 and 2.269g kg^{-1} liveweight for control and yarrow fed birds respectively; P=0.08). Diet dilution had no effect on pancreas weights at 18 days, but at 36 days of age, birds fed the low nutrient density treatment diets had 8% heavier (P=0.014) pancreases than those fed the control treatment diets. Liver weights were also higher for birds fed the low nutrient density treatment diets, both at 18 (P=0.011; table 3.3.7) and 36 (P=0.013; table 3.3.8) days of age. Yarrow fed birds had 5.7% higher (P=0.015) liver weights than controls at 18 days, but this was not observed at 36 days of age.

There were no interactions between yarrow supplementation and diet on liver or pancreas weights at any stage of the experiment.

Table 3.3.8 Proportional liver and pancreas weights of male broilers taken at the end of the grower period (36d) fed control and low nutrient density basal diets with and without yarrow supplementation during the starter and grower phases

Diet	**Performance Variables**	**Yarrow** *Starter/Grower*				**Mean Diet Dilution Effects**
		-/-	-/+	+/-	+/+	
Control	bird weight	2.196	2.129	2.180	2.113	2.155
	liver weight	24.60	26.51	26.67	25.00	25.69
	pancreas weight	2.09	2.03	2.20	2.16	2.12
Low nutrient density	bird weight	2.117	1.966	1.933	2.007	2.006
	liver weight	26.40	26.94	28.20	27.60	27.28
	pancreas weight	2.26	2.21	2.49	2.22	2.29
	Mean Yarrow Effects	-		+		
	bird weight	2.106		2.054		
	liver weight	26.47		26.51		
	pancreas weight	2.26		2.16		

	Statistical significance and SEM of treatment means							
	Diet		**Yarrow**		**Diet x Yarrow Interaction**		**Diet x Y x feeding period Interaction**	
	P	SEM	*P*	SEM	*P*	SEM	*P*	SEM
bird weight	**0.003**	0.0336	NS	0.0336	NS	0.0476	NS	0.0673
liver weight	**0.013**	0.443	NS	0.443	0.063	0.626	NS	0.855
pancreas weight	**0.014**	0.048	NS	0.048	NS	0.068	NS	0.096

NS=P>0.1

As expected, AME values for birds fed low nutrient density diets were significantly lower than those of control fed birds (12.75 *vs.* 11.76MJ kgDM^{-1} for control and low nutrient dense diets respectively; P<0.001). Yarrow supplementation *per se* did not affect AME values (table 3.3.9), but there was an interaction between yarrow and diet: yarrow supplementation enhanced AME by 0.49MJ kgDM^{-1} in control fed birds and reduced it by 0.41MJ kgDM^{-1} in birds fed the low nutrient density diets (P=0.019). Droppings produced by birds fed low

nutrient density diets were higher in dry matter than controls ($P<0.05$, table 3.3.9), but there was no effect of yarrow supplementation or yarrow x diet interaction.

Table 3.3.9 The effect of yarrow supplementation and diet on AME and dry matter values of droppings (birds 24-27 days of age)

Variable	Treatment diet				SEM	Effect of treatment diet		
	Control		Low nutrient density			Diet	Yarrow +/-	Diet x Yarrow
	-	+	-	+			*(P)*	
AME (MJ kg^{-1} DM)	12.50	12.99	11.96	11.55	0.183	**<0.001**	NS	**0.019**
Droppings DM ($g\ kg^{-1}$)	269.5	269.0	290.5	281.4	6.73	**0.017**	NS	NS

NS=$P>0.1$

The grand mean for lipase activity in the small intestinal chyme at 18 days of age was 9.05 units g wet $digesta^{-1}$ (table 3.3.10). This figure is within ranges quoted by previous authors studying lipase activity in broilers (Knarreborg *et al.*, 2003) and rabbits (Al-Mammary *et al.*, 1998), although lower than those quoted in broiler studies by Lee and workers (2003a) and Arija *et al.* (1998). Lipase activity in the small intestine of birds fed low nutrient density diets was 69.2% lower ($P<0.001$) than those fed control diets. In addition, there was an interaction between yarrow supplementation and diet: dietary yarrow increased lipase activity in control fed birds by 26.3%, but reduced it by 22.9% in birds fed low nutrient density diets ($P=0.018$). Lipase activity was more consistent at 36 days of age, with no differences seen between birds fed control and low nutrient density diets, although lipase activity in control fed birds was numerically higher ($P>0.05$).

Table 3.3.10 The effect of yarrow supplementation and diet on lipase activity in small intestinal chyme removed at 18 and 36 days of age (activity expressed as units g wet digesta^{-1})

Diet	Performance Variables	Yarrow				Mean Diet Dilution Effects
		-		+		
Control	18d lipase activity	10.22		12.91		11.56
Low nutrient density	18d lipase activity	7.38		5.69		6.54
	starter/grower	-/-	-/+	+/-	+/+	
Control	36d lipase activity	6.26	8.18	8.67	8.30	7.85
Low nutrient density	36d lipase activity	7.13	6.68	7.14	6.67	6.91
	Mean Yarrow Effects	-		+		
	18d lipase activity	8.80		9.30		
	36d lipase activity	7.30		7.46		

	Statistical significance and SEM of treatment means							
	Diet		**Yarrow**		**Diet x Yarrow Interaction**		**Diet x Y x feeding period Interaction**	
	P	SEM	*P*	SEM	*P*	SEM	*P*	SEM
18d lipase activity	**<0.001**	0.632	NS	0.632	**0.018**	0.894	-	-
36d lipase activity	NS	0.434	NS	0.434	NS	0.613	NS	0.867

NS=*P*>0.1

Amylase activity in the small intestinal chyme taken from birds at 18 and 36 days of age is presented in table 3.3.12. Ranges in activity levels were consistent with those reported by Lee *et al.* (2003a) in 21 and 40 day old broilers and Svihus *et al.* (2004) in 20 and 25 day old broilers. No statistically significant trends as a result of yarrow supplementation or diet were observed, although activity was higher in birds fed low nutrient density diets at 18 days of age ($P>0.05$), and the effects of yarrow supplementation were more prominent in control fed birds than birds fed low nutrient density diets during the starter period ($P>0.05$). However, the range

of activity levels measured was large, hence the high standard error values seen, and the subsequent difficulty in proving any statistically significant effects.

Table 3.3.11 The effect of yarrow supplementation and diet on amylase activity in small intestinal chyme removed at 18 and 36 days of age (activity expressed as units g wet digesta^{-1})

Diet	**Performance Variables**	**Yarrow**				**Mean Diet Dilution Effects**
		-		+		
Control	18d amylase activity	88.4		95.9		92.2
Low nutrient density	18d amylase activity	114.8		115.5		115.2
	starter/grower	-/-	-/+	+/-	+/+	
Control	36d amylase activity	107.2	101.6	96.8	92.2	99.4
Low nutrient density	36d amylase activity	77.9	111.6	108.0	113.6	102.8
	Mean Yarrow Effects	-		+		
	18d amylase activity	101.6		105.7		
	36d amylase activity	97.5		104.7		

	Statistical significance and SEM of treatment means							
	Diet		**Yarrow**		**Diet x Yarrow Interaction**		**Diet x Y x feeding period Interaction**	
	P	SEM	*P*	SEM	*P*	SEM	*P*	SEM
18d amylase activity	NS	17.8	NS	17.8	NS	25.18	-	-
36d amylase activity	NS	9.19	NS	9.19	NS	12.99	NS	18.38

NS=P>0.1

Trypsin activity values in the current study are higher than those reported by Lee *et al.* (2003a) who quoted ranges of 10-16 units g wet digesta^{-1} in 21 day old broilers and 5-20 units g wet digesta^{-1} in 40 day old broilers. During the starter period, yarrow supplementation appeared to have the most profound effects on birds fed the low nutrient density diets, but there was no statistical proof for this (P>0.05). However, birds fed yarrow diets during the starter period tended (P=0.052) to have higher trypsin activity levels than unsupplemented birds at 36 days

of age (31.4 *vs.* 35.4 units g wet digesta^{-1} for control and yarrow fed birds respectively). There was also an interaction between yarrow supplementation during the starter period and diet on trypsin activity at 36 days of age. Birds fed yarrow supplemented control diets up to 18 days of age had higher (P=0.007) levels of trypsin activity at 36 days of age than their unsupplemented conspecifics, but this effect was not seen in birds fed low nutrient density diets.

Table 3.3.12 The effect of yarrow supplementation and diet on trypsin activity in small intestinal chyme removed at 18 and 36 days of age (expressed as units g wet digesta^{-1})

Diet	Performance Variables	Yarrow				Mean Diet Dilution Effects
		-		+		
Control	18d trypsin activity	33.2		34.8		34.0
Low nutrient density	18d trypsin activity	25.6		34.6		30.1
	starter/grower	-/-	-/+	+/-	+/+	
Control	36d trypsin activity	24.5	32.1	42.7	32.8	33.0
Low nutrient density	36d trypsin activity	34.6	34.6	38.3	27.9	33.8
	Mean Yarrow Effects	-		+		
	18d trypsin activity	29.4		34.7		
	36d trypsin activity	35.0		31.8		

	Statistical significance and SEM of treatment means							
	Diet		**Yarrow**		**Diet x Yarrow Interaction**		**Diet x Y x feeding period Interaction**	
	P	SEM	*P*	SEM	*P*	SEM	*P*	SEM
18d trypsin activity	NS	2.32	NS	2.32	NS	3.28	-	-
36d trypsin activity	NS	1.44	NS	1.44	NS	2.03	NS	2.87

NS=P>0.1

Chymotrypsin activity values in the current study are within the ranges reported by Lee *et al.* (2003a) who quoted ranges of 5-7 U g wet digesta^{-1} in 21 day old broilers and 2-7 units g wet

digesta^{-1} in 40 day old broilers. Birds fed the low nutrient density diets showed reduced levels of chymotrypsin activity at the end of both growing periods ($P<0.05$, table 3.3.13). However, chymotrypsin activity was not affected by yarrow supplementation at either 18 or 36 days of age. There was a trend ($P=0.075$) for an interaction between yarrow supplementation and diet during the grower period: birds fed unsupplemented control diets tended to have higher chymotrypsin activity than birds fed yarrow supplemented control diets and birds fed low nutrient density diets.

Table 3.3.13 The effect of yarrow supplementation and diet on chymotrypsin activity in small intestinal chyme removed at 18 and 36 days of age (activity expressed as units g wet digesta^{-1})

Diet	**Performance Variables**	**Yarrow**				**Mean Diet Dilution Effects**
		-		+		
Control	18d chymotrypsin activity	4.01		4.17		4.09
Low nutrient density	18d chymotrypsin activity	3.46		3.20		3.33
	starter/grower	**-/-**	**-/+**	**+/-**	**+/+**	
Control	36d chymotrypsin activity	6.12	4.99	6.16	4.81	5.52
Low nutrient density	36d chymotrypsin activity	4.87	4.08	4.37	5.52	4.71
	Mean Yarrow Effects	-		+		
	18d chymotrypsin activity	3.74		3.69		
	36d chymotrypsin activity	5.38		4.85		

	Statistical significance and SEM of treatment means							
	Diet		**Yarrow**		**Diet x Yarrow Interaction**		**Diet x Y x feeding period Interaction**	
	P	SEM	*P*	SEM	*P*	SEM	*P*	SEM
18d chymotrypsin activity	**0.005**	0.181	NS	0.181	NS	0.256	-	-
36d chymotrypsin activity	**0.042**	0.278	NS	0.278	0.075	0.393	NS	0.556

NS=$P>0.1$

Apparent amino acid digestibility coefficients were high (average 82%) (table 3.3.14), but there was no effect of diet or yarrow supplementation on the digestibility of the combined totals of either indispensable or dispensable amino acids. Although some effects of yarrow supplementation and diet were observed, they were mostly seen in the dispensable amino acids, so their biological significance is questionable. Neither yarrow supplementation nor diet had any effect on the digestibility co-efficients of the three key limiting amino acids, that is methionine, lysine or tryptophan.

Table 3.3.14 Effect of treatment diet on amino acid apparent digestibility co-efficients at 27 days of age

AA digestibility co-efficient	Treatment diet				Main effects		
	Control		Low nutrient density		Yarrow	Diet	Yarrow x Diet Interaction
	-	+	-	+			
Dispensable amino acids							
Asp	0.798	0.757	0.811	0.807	NS (0.0261)	NS (0.0261)	NS (0.0370)
Ser	0.758	0.708	0.777	0.782	NS (0.0094)	** (0.0094)	* (0.1327)
Glu	0.893	0.840	0.904	0.903	* (0.0094)	* 0.0094)	NS (0.1329)
Pro	0.784	0.739	0.772	0.796	NS (0.0326)	NS (0.0326)	NS (0.0460)
Gly	0.944	0.934	0.949	0.948	NS (0.0051)	NS (0.0051)	NS (0.0072)
Tyr	0.715	0.684	0.785	0.779	NS (0.0164)	** (0.0164)	NS (0.0232)
Indispensable amino acids							
His	0.822	0.795	0.859	0.859	NS (0.0060)	** (0.0060)	NS (0.0085)
Thr	0.761	0.718	0.772	0.766	NS (0.0222)	NS (0.0222)	NS (0.0314)
Arg	0.873	0.849	0.859	0.852	NS (0.0201)	NS (0.0201)	NS (0.0284)
Val	0.778	0.725	0.799	0.784	NS (0.0172)	NS (0.0172)	NS (0.0243)
Phe	0.690	0.632	0.737	0.705	NS (0.0308)	NS (0.0308)	NS (0.0435)
Iso	0.704	0.643	0.754	0.749	NS (0.0362)	NS (0.0362)	NS (0.0511)
Try	0.794	0.773	0.818	0.796	NS (0.0230)	NS (0.0230)	NS (0.0325)
Met	0.960	0.951	0.959	0.956	NS (0.0090)	NS (0.0090)	NS (0.0127)
Cys	0.741	0.702	0.755	0.765	NS (0.0245)	NS (0.0245)	NS (0.0347)
Leu	0.698	0.630	0.722	0.721	NS (0.0454)	NS (0.0454)	NS (0.0642)
Lys	0.867	0.847	0.899	0.873	NS (0.0208)	NS (0.0208)	NS (0.0294)
Dis AA	0.815	0.833	0.777	0.836	NS (0.0138)	NS (0.0138)	NS (0.0195)
Indis AA	0.790	0.751	0.812	0.802	NS (0.0222)	NS (0.0222)	NS (0.0313)
Total AA	0.799	0.760	0.819	0.814	NS (0.0191)	NS (0.0191)	NS (0.0270)

NS=P>0.05; *=P<0.05; **=P<0.01; Values in parenthesis are the SEM

3.3.5 Discussion

Analysis of the essential oil distilled from the yarrow herb material used in this series of experiments has revealed that the major components found are consistent with those reported in the literature. In addition, the amounts of each chemical component appear to be within reported ranges with the exception of 1,8-cineole (slightly low) and borneol (high). However, the chemical composition of herbs can be highly variable as a result of differences in growing location, season of collection and processing methods. The components identified belong to the terpenoid class of compounds, and are mainly monoterpenes, which have antimicrobial properties, and sesquiterpenes, which have antimicrobial and 'bitter' properties.

Other researchers working in the area of herbal supplementation for broiler chickens have postulated that feeding highly digestible diets may mask the beneficial effects of the herbs (de Freitas *et al.*, 2001; Botsoglou *et al.*, 2002; Cross *et al.*, 2003; Lee *et al.*, 2003a; Hernandez *et al.*, 2004; Jang *et al.*, 2004), and have suggested that reducing diet quality may enhance the effects of herbal supplementation. The following experiment was, in part, designed to test the hypothesis that the growth performance effects of yarrow supplementation would be more prominent in birds fed low nutrient density diets than birds fed control diets formulated to meet nutritional requirements for the growing broiler.

Experimental low nutrient density diets were formulated to be high in fibre, in order to depress growth performance (Annison, 1990; Choct and Annison, 1990; Smits *et al.*, 1997; Smits *et al.*, 1998). Indeed, daily liveweight gains of birds fed the less nutrient dense high fibre diets were approximately 10 and 6% lower than control fed birds in the starter and grower feeding periods respectively. This concurs with the diet dilution work carried out by Leeson *et al.*

(1992) who found that the reduction in weight gain (as a percentage of the control) diminished with increasing age, indicating some sort of adaptation to diet dilution. He reasoned that this adaptation was a result of increased feed intakes in response to the lower energy density of the diet. This is in contrast to the findings of the current study where diet did not affect feed intake at any stage. However, there was a trend (P=0.087) for a diet x yarrow supplementation interaction during the grower period where birds fed yarrow supplemented low nutrient dense diets ate nearly 6% more feed than those fed unsupplemented low nutrient density diets. Nevertheless, the concomitant increased growth rates, although numerically superior, were not statistically proven (P>0.05).

Although no direct yarrow mediated growth performance benefits were noted, some interesting feeding period x yarrow supplementation interactions were observed. Contrary to expectations, the response to dietary yarrow was greater in birds fed control diets than that of birds fed low nutrient density diets. Overall performance results (0-36d) indicate that yarrow supplementation of control diets during the starter period led to lower daily feed intakes compared with unsupplemented controls (P<0.05) with a tendency for increased overall FCE (P=0.069).

The number of controlled studies on the effect of herbs on digestive enzymes in broiler chickens is very limited. However, the few scientific papers available suggest that herbs and essential oils stimulate the activity of digestive enzymes in chickens (Williams and Losa, 2001; Lee *et al.*, 2003a; Jang *et al.*, 2004) and rats (Platel and Srinivasan, 1996; Platel and Srinivasan, 2000). There is also anecdotal evidence to suggest that the bitter components present in yarrow may stimulate digestive enzyme secretion and subsequent nutrient digestibility (Chandler, 1992a; Leung and Foster, 1996; Moerman, 1997; Hoffman, 1988;

McCartney, 2002). It was therefore hypothesised that yarrow supplementation may result in increased digestive enzyme activity, thus in the present study, activity levels of amylase, lipase, trypsin and chymotrypsin in the small intestinal chyme were examined. Neither yarrow or diet dilution had any effect on amylase activity at any stage of the experiment, which eliminates any theory that the increase in AME observed in birds fed yarrow supplemented control diets is a result of improved starch digestion. Although some yarrow mediated effects were observed on trypsin and chymotrypsin activity, the lack of any statistical improvements on amino acid digestibility indicates that there were no biologically significant beneficial effects on protein digestion. Lipase activity was not affected by yarrow supplementation *per se*, but there was an interaction between yarrow and diet dilution which was also observed in dietary AME: yarrow supplementation increased both AME and 18d lipase activity in control diets, but reduced both these variables in low nutrient dense diets ($P<0.05$). The reason why yarrow reduced lipase activity in low nutrient density diets is unclear. It may be that the increased bulk of digesta present in the digestive tract as a result of feeding high fibre diets diluted the endogenous enzymes, thus reducing the chance of detecting statistical differences. Or it may be as a result of the anti-nutritional effects of feeding high fibre diets. Studies by Knarreborg and workers (2003) have demonstrated that lipase activity in the small intestine is proportional to pH. It is reasonable to suggest that feeding high fibre diets would lead to increased fermentation and thus decreased pH in the enteric environment, which may in part explain the lower lipase activity in the small intestinal chyme of birds fed the low nutrient density diets. It is also known that the sensitivity of broilers to the anti-nutritional factors present in fibre decreases with increasing age (Leeson *et al.*, 1992; Leeson *et al.*, 1996), which may explain the lack of diet effect on lipase activity at 36 days of age.

The interaction between yarrow supplementation of control diets during the starter period and overall growth performance during the experimental period indicates an improvement in efficiency, as yarrow fed birds ate less ($P<0.05$) but achieved equivalent weight gains ($P>0.05$) relative to their non-supplemented conspecifics, with a tendency for improved FCE ($P=0.069$). The reduced feed intake of broilers fed yarrow supplemented diets during the starter period may be interpreted as a direct manifestation of improved feed quality. It is possible that the improved nutritive quality of these diets satiated the chickens with lower feed intakes. This theory is supported by the similar pattern observed in lipase activity in the small intestinal chyme, where yarrow supplementation of control diets led to elevated ($P<0.05$) lipase activity during the starter period. This may explain the higher dietary AME values observed in chickens fed yarrow supplemented control diets. Noy and Sklan (1995) reported that lipase activity increased at a slower rate post-hatch than other enzymes making fat digestion a limiting factor in early growth performance. It therefore seems probable that the growth promoting factor of yarrow (in highly digestible diets) reflects increased efficiency in the transformation of exogenous nutrients, which is likely achieved through increased lipase activity during the starter period and subsequent improvements in dietary fat utilisation.

3.4 THE EFFECTS OF YARROW SUPPLEMENTATION ON GROWTH PERFORMANCE AND NUTRIENT DIGESTIBILITY IN CHICKENS FED DIETS CONTAINING DIFFERENT FAT SOURCES

3.4.1 Introduction

Results from the previous study indicate that the likely mode of action for yarrow involves an improvement in the utilisation of dietary fats. Dietary fats provide a large proportion of the energy in poultry feeds, thus efficient fat digestion is crucial for growth. Fat digestion in poultry is influenced by numerous factors, of which age of bird and fat source are most reported (Sell *et al.*, 1986; Wiseman and Salvador, 1991; Leeson and Atteh, 1995; Daenicke *et al.*, 2000; Meng *et al.*, 2004). The digestive systems of young birds are immature at hatch (Sell *et al.*, 1986), and some essential digestion components are sub-optimally supplied during the first weeks of life, particularly lipase and bile salts (Krogdahl and Sell, 1989). Noy and Sklan (1995) reported that lipase activity increased at a slower rate post-hatch than other enzymes making fat digestion a limiting factor in early growth performance. This age-related effect on fat utilisation is also correlated with low levels of bile salt production in young chicks (Serafin and Nesheim, 1970). Studies have shown that dietary addition of bile salts improves the utilisation of dietary fat (Eyssen *et al.*, 1965; Atteh and Leeson, 1985), but synthetic bile salts are too costly to be commercially viable. Thus it is normally accepted that diets for young chicks should be based on highly digestible ingredients in order to maximise the efficiency of digestion, but this increases the cost of the diet.

The previous study demonstrated an increase in lipase activity following yarrow supplementation of highly digestible basal diets. It is hypothesised that this yarrow mediated

increase in lipase activity may be more profound when diets containing poorly digestible fats are fed, as several published studies have demonstrated that exogenous lipase and bile salt supplementation improves growth performance and fat digestibility, particularly in diets containing saturated fatty acids (Eyssen *et al.*, 1965; Gomez and Polin, 1976; Polin *et al.*, 1980; Polin and Hussein, 1982). This could have wide economic implications for broiler nutrition during the early growth period, allowing formulators to utilise alternative, cheaper fat sources without a concomitant reduction in broiler performance.

3.4.2 Specific Objectives

The aim of this experiment was to investigate the effects of yarrow supplementation and its interaction with fat source. The specific objectives were to examine:

i. growth performance of caged male broilers fed one of eight treatment diets in a 2x4 factorial design (four fat sources with and without yarrow supplementation)
ii. digestive enzyme activity and bile acid concentrations
iii. nutrient digestibility

3.4.3 Material and Methods

Feed additives

The yarrow premix was provided by Braes Feed Ingredients (Chester, UK), and was from the same batch as those used in previous experiments.

Dietary Treatments

Caged male broilers (10-20 days of age) were offered one of eight treatment diets (Table 3.4.1), which differed in fat source and yarrow supplementation. Three sources of fat were added to the basal (control) diet at 50g kg^{-1}. Fat sources used were soya bean oil, crude palm oil and palm oil fatty distillate, which differed in degree of saturation and free fatty acid content (table 3.4.3).

Table 3.4.1 Treatment Diets

Treatment diet	Fat source	Yarrow
C-	None	-
C+	None	+
SBO-	Soya bean oil *	-
SBO+	Soya bean oil *	+
CPO-	Crude palm oil *	-
CPO+	Crude palm oil *	+
POFD-	Palm oil fatty distillate *	-
POFD+	Palm oil fatty distillate *	+

C=Control; SBO=Soya bean oil; CPO=Crude palm oil; POFD=Palm oil fatty distillate; * included at 50g kg^{-1}
- = no yarrow; + = yarrow

Palm oil is the world's second most important vegetable oil after soyabean oil, and currently accounts for 13% of the world's total production of oils and fats (Sambanthamurth *et al.*, 2000). It is derived from the flesh of the fruit from the *Elaeis* species, a perennial tree crop which yields around 3.7t of crude palm oil per hectare per year in Malaysia (Sambanthamurth *et al.*, 2000). Crude palm oil is refined (through steam refining, degumming and deacidification) to yield products suitable for a range of applications including human food, animal feed, plastics and soaps (Malaysian Palm Oil Promotional Council, 2005). Palm oil fatty distillate is a biproduct of the deacidification process, and is a low value waste product (Sambanthamurth *et al.*, 2000). It contains higher concentrations of saturated fatty acids and free fatty acids than crude palm oil (Sambanthamurth *et al.*, 2000). Ingredient composition and calculated analysis of the four basal diets is shown in table 3.4.2.

Table 3.4.2 Ingredient composition and calculated analysis of experimental diets

Feedingstuff	**Inclusion rate (kg tonne^{-1})**			
	C	**SBO**	**CPO**	**POFD**
Wheat	658	625	625	625
Hipro soya bean meal	250	238	238	238
Maize gluten meal	42.2	40	40	40
Soya oil	-	50	-	-
Palm oil	-	-	50	-
Palm oil fatty distillate	-	-	-	50
Lysine HCl	3.2	3	3	3
Methionine	2.6	2.4	2.4	2.4
Limestone	5.3	5	5	5
Dicalcium phosphate	8.4	8	8	8
Salt	3.8	3.6	3.6	3.6
Vitamin and mineral premix [1]	21.2	20	20	20
Herbal product/dextrose control	5.3	5	5	5
Total	**1000**	**1000**	**1000**	**1000**
Calculated analysis				
Nutrient	**Concentration (per kg dry matter [2])**			
Metabolisable Energy	13.0 MJ	14.8 MJ	14.0 MJ	13.5 MJ
Crude fat	46.2	108.1	110.6	106.4
Crude protein	223.9	212.8	212.8	212.8
Crude fibre	27.6	26.3	26.3	26.3
Lysine	12.78	12.13	12.13	12.13
Methionine and cystine	9.68	9.14	9.14	9.14
Calcium	9.32	8.83	8.83	8.83
Phosphorus	5.56	5.30	5.30	5.30
Sodium	1.86	1.77	1.77	1.77

[1] The vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by NRC (1994). The major components were: phosphorus, 95g/kg; calcium, 219g/kg; sodium, 30g/kg; copper sulphate, 0.5g/kg; selenium, 10mg/kg; retinol acetate, 0.275g/kg; cholecalciferol, 625mg/kg; alpha tocopherol, 2.273g/kg. The product was sourced from Ian Hollows Feed Supplements, Whitchurch, Shropshire.

[2] Calculated dry matter 868mg kg^{-1} for C diets and 874mg kg^{-1} for SO, CPO and POFD diets.

Diet Manufacture

The feed was manufactured at Harper Adams University College. Experimental diets were mixed in 25kg batches according to treatment diet specifications. Appropriate concentrations of fat and either yarrow product or dextrose (control) were added to the basal diet and mixed in a horizontal mixer for 3 minutes to ensure a homogenised mixture. Feed was presented in mash form.

Animal Husbandry

A total of 216 male broiler chickens were obtained from a commercial hatchery (Maurice Millard, Trowbridge) at day old and placed in a communal floor pen on wood shavings. Chicks were offered a commercial wheat and soya based starter diet (Laser SP starter crumbs, BOCM Pauls Limited) containing 205g kg^{-1} crude protein. At 10 days of age birds were randomly assigned to cages in groups of three, with each cage serving as a treatment replicate. The cages were housed in an environmentally-controlled metabolism room. There were 72 cages in total, thus nine replicates per treatment diet. Cages were arranged in three tiers. Birds were given free access to feed and water at all times. The temperature was maintained initially at 26°C but was reduced by 1°C daily to 22°C. An hour of darkness was provided daily.

Growth Performance Determination

Performance data were collected on a cage basis. Birds were weighed when placed in the cages, and at the beginning and end of the AME collection period. Feed consumption was also determined over these two time periods.

Dissections

At the end of the experiment (birds 20 days old) 2 birds per cage were randomly selected and killed by cervical dislocation. Birds were individually weighed and dissected. Pancreas weights were recorded. Digesta samples were obtained by gentle finger stripping of the appropriate intestinal segments, as described previously (section 3.3.3). Digesta were pooled from both birds per cage to ensure that a sufficient amount of digesta were obtained. Gizzard contents were removed and weighed. Digesta and gizzard contents were rapidly frozen, and stored at –20°C pending laboratory analysis.

Laboratory Analysis

Fatty Acid Analysis of Feed Samples

The feed fatty acid profiles of the samples were determined by gas liquid chromatography according to the method described by Wachira *et al.* (2002). After addition of the fatty acid standard, heneicosanoic acid methyl ester (Sigma-Aldrich, UK), the solvents were removed under nitrogen and the lipids hydrolysed with 2M potassium hydroxide in methanol-water (1:1 v/v) containing 1g hydroquinone l^{-1} as antioxidant, at 60°C for one hour. After dilution with water and removal of non-saponifiable compounds with three extractions with petroleum ether, the hydrolysate was acidified and the fatty acids extracted. The fatty acids were methylated with a solution of diazomethane in diethyl ether and their composition compared using as gas liquid chromatograph (PerkinElmer 8500; PerkinElmer Life and Analytical Sciences Ltd., Boston, USA). The chromatograph had attached a Perkin Elmer AS 8300 autosampler and utilised helium as the carrier gas, split 70:1. The identification of the different fatty acids was carried out by comparison with retention times of known pure fatty acid standards (Sigma-Aldrich, UK).

Determination of Digestive Enzyme Activity and Bile Acid Concentrations

Intestinal duodenum plus jejenum contents were individually homogenised in 4 volumes of ice-cold distilled water and centrifuged at 6500 x *g* for 15 minutes at 4°C (Lee *et al.*, 2003a). Aliquots of the supernatant were removed for determination of amylase (EC 3.2.1.1) and lipase (EC 3.1.1.3) activity (as described in section 3.3.3), and bile acid concentrations. Bile acid concentrations were measured on a Cobas Mira blood analyser (ABX Diagnostics, Montpellier, France) using a Randox test kit (Randox catalogue number BI 1689). Aliquots of gizzard contents were prepared in the same way and analysed for bile acid concentrations.

AME Determination

Apparent Metabolisable Energy (AME) was determined using a total collection method (Sibbald, 1989) over four days (birds 16-20 days of age). Representative homogenised droppings samples (500g) were oven-dried at 60°C for 48 hours, and dry matter content calculated. The gross energy of each dried droppings sample and the experimental diets were determined using a Parr 1261 adiabatic bomb calorimeter (Parr Instrument Company, USA). The AME content of the eight diets was calculated by deducting the amount of energy contained in the collected droppings from the energy intakes of the birds over the four-day period.

Fat Digestibility

Droppings collected during the AME experiment were freeze dried (Model B, series 6/13; Girovac Limited, Hertfordshire) and ground to pass through a 0.25mm screen. Crude fat content of experimental diets and droppings was measured according to standard AOAC (2000) methods (procedure number 920.39) using the Soxtec system. Briefly, 3g of sample was weighed into thimbles and boiled in petroleum ether for one hour. After 30 minutes of

rinsing, condensed petroleum ether was allowed to collect in the pre-weighed extraction cups. Once the petroleum ether had evaporated the pre-weighed extraction cups were re-weighed to determine the amount of fat liberated from the sample. The fat content was calculated as:

Ether Extract (g $kgDM^{-1}$) = (weight of fat (g)/weight of sample (g)) * 1000

Statistical Analysis

Cage of birds was considered the experimental unit. The data obtained were compared by ANOVA using the Genstat program (Genstat 5; Lawes Agricultural Trust). The level of statistical significance was pre-set at $P<0.05$, although any probability level lower than 0.1 has been indicated.

3.4.4 Results

The fatty acid content of the experimental diets is shown in table 3.4.3.

Table 3.4.3 Fatty acid amounts in the experimental diets (mg fatty acid g feed^{-1})

Fatty acid	Diet			
	None	Soya Bean Oil	Crude Palm Oil	Palm Oil Fatty Distillate
Saturated Fatty Acids				
C10:0 (Capric)	0.0	0.0	0.1	0.1
C11:0	0.0	0.0	0.2	0.2
C12:0 (Lauric)	0.0	0.0	0.3	0.2
C13:0	0.0	0.0	0.0	1.0
C14:0 (Myristic)	0.1	0.1	1.0	1.1
C15:0	0.0	0.1	0.0	0.0
C16:0 (Palmitic)	7.8	14.2	36.2	37.3
C17:0 (Margaric)	0.1	0.1	0.3	0.2
C18:0 (Stearic)	0.8	3.0	3.3	3.3
C20:0 (Arachidic)	0.0	0.8	0.4	0.4
C21:0	0.1	0.5	0.1	0.0
C23:0	0.0	0.0	0.5	1.1
C24:0	0.7	0.1	0.1	0.0
Unsaturated Fatty Acids				
C16:1 (Palmitoleic)	0.0	0.1	0.0	0.0
C17:1	0.0	0.1	0.0	0.0
C18:1 (Oleic)	3.9	16.7	33.3	30.3
C18:2 (Linoleic)	27.7	63.0	31.0	28.5
C18:3 (Linolenic)	3.2	7.9	2.4	2.2
C20:1 (Gladoleic)	0.2	0.4	0.2	0.2
C22:1 (Erucic)	0.2	0.2	0.1	0.0
C22:2	0.1	0.0	0.0	0.0
Σ Total Saturated Fatty Acids	*9.6*	*18.9*	*42.5*	*44.6*
Σ Total Unsaturated Fatty Acids	*35.3*	*88.4*	*67.0*	*61.2*
Proportion of Saturated Fatty Acids	**214**	**176**	**387**	**423**
Proportion of Unsaturated Fatty Acids	**786**	**824**	**613**	**577**
Proportion of Free Fatty Acids [§]	**-**	**9**	**100**	**886**

[§] Expressed as a proportion of fat prior to dietary inclusion, values provided by suppliers

The proportion of free fatty acids in each fat source has been indicated. As expected, diets containing crude palm oil and palm oil fatty distillate contained higher levels of saturated fats

than diets containing soya bean oil or no added fat. The fat sources comprised a wide range of free fatty acid content, with palm oil fatty distillate containing the highest amount, and soya bean oil containing the least.

There was no mortality during the experimental period. Daily weight gain, daily feed intake and feed conversion efficiency data for the experimental period are presented in table 3.4.4. Daily weight gains were significantly affected by fat source ($P<0.001$), with birds fed diets containing soya bean oil achieving the highest weight gains. Weight gains for control, crude palm oil and palm oil fatty distillate fed birds were 8.9, 17.6 and 5.8% lower respectively relative to those fed soya bean oil. Feed intake was also influenced by fat source ($P=0.007$): birds fed diets containing palm oil fatty distillate ate more than birds fed the other treatment diets. The lowest feed intakes were seen in birds fed dietary treatments containing crude palm oil. Accordingly, birds fed diets containing soya bean oil had higher FCE ($P<0.001$) than birds fed treatment diets containing the other fat sources, and birds fed crude palm oil diets achieved the lowest FCE ($P<0.001$).

Daily feed intake was not affected by yarrow supplementation, although yarrow fed birds tended ($P=0.061$) to eat more than their unsupplemented conspecifics. Yarrow supplementation improved daily weight gains ($P<0.001$) by nearly 11% and FCE ($P<0.001$) by nearly 9% during the experimental period. In addition, there were interactions between fat source and yarrow supplementation on weight gain ($P=0.002$), feed intake ($P=0.036$) and FCE ($P=0.002$). Growth performance of birds fed diets containing no added fat or soya bean oil were unaffected by yarrow supplementation, but yarrow supplementation of diets containing crude palm oil and palm oil fatty distillate improved growth rates by 23 and 18% respectively and increased feed intake by 10 and 3% respectively in comparison to non-yarrow

supplemented controls. In congruence with these findings, FCE was unaffected in birds fed diets containing no added fat or soya bean oil, but improved by some 15% in birds fed diets containing crude palm oil and palm oil fatty distillate (P=0.002). Growth rates achieved by birds fed yarrow supplemented diets containing palm oil fatty distillate were the highest recorded during the experimental period, and were higher than mean growth rates achieved for birds fed diets supplemented with soya bean oil.

Table 3.4.4 Effect of dietary fat source and yarrow supplementation on individual bird daily weight gain, daily feed intake and FCE (10-21d)

Fat Source	Yarrow -	Yarrow +	Mean Fat Source Effects
Daily Liveweight Gain (g day^{-1})			
C	36.60	35.93	36.26
SBO	39.27	40.36	39.82
CPO	28.41	37.14	32.78
POFD	34.16	41.65	37.91
Mean Yarrow Effects	34.61	38.77	
Daily Feed Intake (g day^{-1})			
C	65.76	64.49	65.12
SBO	66.47	66.68	66.57
CPO	60.59	67.59	64.09
POFD	68.11	70.05	69.08
Mean Yarrow Effects	65.23	67.20	
FCE (gain : feed)			
C	0.556	0.555	0.556
SBO	0.589	0.605	0.597
CPO	0.468	0.549	0.508
POFD	0.500	0.592	0.546
Mean Yarrow Effects	0.528	0.575	

	Statistical significance and SEM of treatment means					
	Yarrow		**Fat Source**		**Yarrow x Fat Source**	
	P	SEM	*P*	SEM	*P*	SEM
DLWG	**<0.001**	0.707	**<0.001**	1.000	**0.002**	1.414
DFI	0.061	0.073	**0.007**	1.030	**0.036**	1.457
FCE	**<0.001**	0.0069	**<0.001**	0.0098	**0.002**	0.01395

There were significant effects of fat type on diet AME content ($P<0.001$), fat digestibility ($P<0.01$) and dry matter digestibility ($P<0.01$) (Table 3.4.5). Birds fed diets containing soya bean oil achieved the highest AME ($P<0.001$) and dry matter digestibility co-efficients ($P<0.01$), whilst birds fed diets containing no added fat attained the lowest AME values and those fed diets containing the palm oil fatty distillate the lowest dry matter digestibility co-efficients. Fat digestibility co-efficients varied from 0.46 to 0.70. Diets containing soya bean oil resulted in the highest fat digestibility co-efficients ($P<0.01$), with the groups receiving no supplementary fat achieving the lowest fat digestibility. Chickens fed on diets containing soya bean oil digested approximately 17 and 15% more fat than chickens given diets containing the crude palm oil and palm oil fatty distillate respectively.

The main effects of yarrow supplementation did not affect ($P>0.05$) AME values or fat or dry matter digestibility co-efficients, nor were there any interactive effects observed between yarrow supplementation and fat source.

Table 3.4.5 Effect of dietary fat source and yarrow supplementation on AME, dry matter digestibility co-efficients and fat digestibility co-efficients

Fat Source	Yarrow -	Yarrow +	Mean Fat Source Effects
AME			
C	11.2	11.4	11.3
SBO	12.9	13.0	12.9
CPO	12.3	12.1	12.2
POFD	11.7	11.9	11.8
Mean Yarrow Effects	12.0	12.1	
Dry matter digestibility co-efficients			
C	0.669	0.681	0.675
SBO	0.711	0.717	0.714
CPO	0.698	0.686	0.692
POFD	0.679	0.661	0.669
Mean Yarrow Effects	0.689	0.686	
Fat digestibility co-efficients			
C	0.462	0.534	0.498
SBO	0.626	0.703	0.664
CPO	0.586	0.515	0.551
POFD	0.584	0.544	0.564
Mean Yarrow Effects	0.565	0.574	

	Statistical significance and SEM of treatment means					
	Yarrow		**Fat Source**		**Yarrow x Fat Source**	
	P	SEM	*P*	SEM	*P*	SEM
AME	NS	0.14	**<0.001**	0.19	NS	0.28
Dry matter	NS	0.0093	**0.005**	0.0132	NS	0.0186
Fat	NS	0.0226	**0.005**	0.0320	NS	0.0452

NS=*P*>0.1

Activity of digestive enzymes and bile acid concentrations in the small intestinal chyme are presented in table 3.4.6. Fat source significantly affected amylase activity (P=0.001) and bile salt concentrations (P<0.001), and had a tendency (P=0.066) to influence lipase activity. Amylase activity and bile acid concentrations were highest in birds fed diets with no supplementary fat, and lowest in birds fed diets containing crude palm oil. Conversely, lipase activity was highest in birds fed diets containing soya bean oil and lowest in diets containing no added fat. Lipase activity in birds fed diets containing added fat appears to be positively

correlated with unsaturated dietary fat and/or negatively correlated with dietary free fatty acids.

Yarrow supplementation did not affect digestive enzyme activity or bile salt concentration ($P>0.05$), although they were numerically higher in birds fed diets containing yarrow. There was a trend ($P=0.079$) for an interaction between fat source and yarrow supplementation when lipase activity was expressed as total activity in the tract: yarrow supplementation had no effect on lipase activity in birds fed soya bean oil and crude palm oil diets, but increased lipase activity by approximately 26% and decreased it by approximately 28% in birds fed diets containing palm oil fatty distillate and no added fat respectively. The same numerical trend was observed when expressed as activity per gram of wet chyme, but was not statistically proven ($P>0.1$).

Table 3.4.6 The effect of dietary fat source and yarrow supplementation on lipase and amylase activity and bile acid concentrations in the small intestine

Fat Source	Yarrow -	Yarrow +	Mean Fat Source Effects
Lipase Activity (units g^{-1} wet chyme)			
C	14.46	11.54	13.00
SBO	16.00	17.55	16.78
CPO	16.90	15.45	16.17
POFD	11.81	15.89	13.85
Mean Yarrow Effects	14.79	15.11	
Amylase Activity (units g^{-1} wet chyme)			
C	135.9	161.8	148.8
SBO	96.5	107.1	101.8
CPO	60.7	68.9	64.8
POFD	137.0	121.2	129.1
Mean Yarrow Effects	107.5	114.7	
Bile Acid Concentrations (mg g^{-1} wet chyme)			
C	3.019	3.925	3.472
SBO	3.173	3.005	3.089
CPO	1.777	1.662	1.720
POFD	2.621	2.743	2.682
Mean Yarrow Effects	2.648	2.834	

	Statistical significance and SEM of treatment means					
	Yarrow		**Fat Source**		**Yarrow x Fat Source**	
	P	SEM	*P*	SEM	*P*	SEM
Lipase	NS	0.803	0.066	1.136	NS	1.607
Amylase	NS	10.46	**0.001**	14.79	NS	20.92
Bile Acids	NS	0.1374	**<0.001**	0.1943	NS	0.2747

NS=*P*>0.1

Bile acid concentrations in the gizzard are presented as mg bile acids per gram of wet gizzard contents (Table 3.4.7). Gizzard bile acid concentrations were affected by fat source, with the highest ($P<0.001$) levels seen in birds fed diets containing soya bean oil. The lowest concentration of gizzard bile acids was seen in birds fed diets containing crude palm oil. Yarrow supplementation elevated bile acid concentrations by 29% ($P=0.009$) across all dietary treatments. The biggest effect, which equated to an increase of approximately 82%, was seen in birds fed diets containing palm oil fatty distillate. There was a highly significant interaction ($P=0.006$) between yarrow supplementation and fat source. This was largely as a result of the large (+82%) increase in bile acids concentrations in palm oil fatty acid diets with yarrow supplementation, but yarrow supplementation also increased bile acids in birds fed crude palm oil (+27%). No significant effects were observed as a result of yarrow supplementation in birds fed diets containing soya bean oil or no added fat.

Table 3.4.7 The effect of dietary fat source and yarrow supplementation on bile acid concentrations in the gizzard (mg g^{-1} wet weight)

Fat Source	**Yarrow -**		**Yarrow +**		**Mean Fat Source Effects**	
C	0.286		0.271		0.278	
SBO	0.380		0.392		0.386	
CPO	0.160		0.220		0.190	
POFD	0.064		0.369		0.217	
Mean Yarrow Effects	0.222		0.313			
	Statistical significance and SEM of treatment means					
	Yarrow		**Fat Source**		**Yarrow x Fat Source**	
	P	SEM	*P*	SEM	*P*	SEM
	0.009	0.0231	**<0.001**	0.0326	**0.006**	0.0462

3.4.5 Discussion

The present study has clearly demonstrated that the growth performance of birds fed wheat-based diets was affected by fat type, and that chicks fed diets supplemented with fat sources high in saturated fatty acids, and in particular crude palm oil, had poorer weight gains and gain to feed ratio. This is consistent with the observations of others (Daenicke *et al.*, 1997b; Daenicke *et al.*, 2000; Preston *et al.*, 2001; Meng *et al.*, 2004) who report that feeding saturated fat reduces chick growth performance. In accordance with the results of these studies, AME values were significantly ($P<0.001$) affected by fat source, with diets containing crude palm oil and palm oil fatty distillate having lower values than soya bean oil. In addition, there was a marked reduction in AME values for diets containing the palm oil fatty distillate when compared to those containing crude palm oil (11.8 *vs.* 12.2 MJ $kgDM^{-1}$ for palm oil fatty acid distillate and crude palm oil respectively, SEM 0.194). This is consistent with previous studies investigating the nutritive value of fats and their hydrolysed products (Renner and Hill, 1961; Garrett and Young, 1975; Wiseman and Salvador, 1991; Wiseman and Blanch, 1994; Blanch *et al.*, 1995; Zumbado *et al.*, 1999) that show the reduction in dietary AME corresponds to an increase in free fatty acid concentration in the diet, an effect which is more pronounced with increasing saturation. These authors proposed that the reduction in AME was a result of insufficient monoglycerides and bile salts present in the intestinal lumen resulting in incomplete micellar solubilisation of free fatty acids.

As expected, fat digestibility was affected by fat source ($P<0.01$), with birds fed diets containing soya bean oil achieving higher fat digestibility co-efficients that those fed diets containing crude palm oil or palm oil fatty distillate. This is in agreement with numerous studies (Daenicke *et al.*, 1997b; Langhout *et al.*, 1997; Preston *et al.*, 2001; Meng *et al.*, 2004)

comparing fat digestibility co-efficients in broilers fed predominantly unsaturated and predominantly saturated fat sources. Reported values for fat digestibility in the current study are, on average, lower than those reported by Meng *et al.* (2004) who reported apparent digestibility values of 0.688 to 0.727 for diets containing 50g kg^{-1} tallow and canola oil respectively. However, the birds used in their study were slightly older, which may explain the higher digestibility of fats seen. Other workers investigating the effects of saturated dietary fatty acids on fat digestibility co-efficients have reported values in the range of 0.452–0.782 (Daenicke *et al.*, 1997b), 0.365–0.858 (Daenicke *et al.*, 2000) and 0.55-0.86 (Preston *et al.*, 2001), which are consistent with those seen in the current study (0.462-0.703; table 3.4.9). Dry matter digestibility co-efficients reported in this experiment are consistent with those previously published for broilers (Agunbiade, 2000; Ravindran *et al.*, 2000) and layers (Lazaro *et al.*, 2003). As expected, dry matter digestibility was affected by fat source ($P<0.01$), with digestibility decreasing with increased dietary saturation.

Yarrow supplementation had a positive effect on growth performance, with the most profound improvements noted in birds fed diets containing the higher levels of saturated fatty acids. Dietary yarrow improved weight gains ($P<0.01$) by 23 and 18% and increased feed intake ($P<0.05$) by 10 and 3% in diets containing crude palm oil and palm oil fatty distillate respectively, with an approximate 15% improvement ($P<0.01$) in FCE. Indeed, yarrow supplementation of diets containing palm oil fatty distillate improved bird weight gain and feed conversion efficiency such that results were equivalent to those seen in birds fed diets supplemented with soya bean oil. When considering these important improvements in growth performance, the lack of statistical improvement in fat digestibility as a result of yarrow supplementation was very surprising. Indeed, it appears form the results that yarrow supplementation reduces fat digestibility in these diets, although this was not statistically

proven, even as a trend (table 3.4.5; $P>0.1$). It must be noted, however, that the fat digestibility measurements conducted in the present study were crude and did not include acid hydrolysis prior to solvent extraction. It would appear that the fat digestibility results are spurious, and on this basis, data pertaining to dry matter digestibility offers a more realistic guide to overall nutrient availability. However, yarrow supplementation did not affect dry matter digestibility, which implies that yarrow does not increase the availability or assimilation of dietary nutrients. This observation is supported further by the lack of statistical effects of yarrow supplementation on dietary AME.

It was hypothesised, based on the results of the previous experiment, that dietary yarrow would increase lipase activity in the small intestinal lumen with a concomitant increase in fat digestibility values. It was thought that the response would be greater in birds fed crude palm oil and palm oil fatty acid distillate, as several published studies (Eyssen *et al.*, 1965; Gomez and Polin, 1976; Polin *et al.*, 1980; Polin and Hussein, 1982) have demonstrated that exogenous lipase and bile salt supplementation improves fat digestibility, particularly in diets containing high levels of saturated fatty acids. However, unlike the previous experiment, there was no effect of yarrow supplementation on lipase activity in the small intestinal chyme. There tended ($P=0.079$) to be an interaction between fat source and yarrow supplementation on lipase activity (table 3.4.6) where yarrow supplementation elevated lipase activity in birds fed diets containing palm oil fatty distillate, but reduced levels in diets not containing added fat.

Yarrow supplementation increased gizzard bile salt concentrations ($P=0.009$), particularly in diets containing higher levels of saturated fat ($P=0.006$). Bile salts are essential for the emulsification of fats and activation of lipase, especially for saturated fatty acids (Johnston,

1977) as opposed to short chain and unsaturated fatty acids which are more easily absorbed even in the absence of bile salts (Garrett and Young, 1975). Since bile acids enter the intestine through the proximal duodenal loop, their levels in the gizzard contents give a good indication of gastrointestinal reflux (Hetland and Choct, 2003). Reflux is the retrograde movement of digesta in the gastrointestinal tract, and is likely to be controlled by cholecystokinin (CCK) levels. CCK is produced mainly in the duodenal region, and acts through the vagus nerve to stimulate gastrointestinal reflux (Duke, 1992; Svihus *et al.*, 2004) and digestive enzyme secretion (Denbow, 2000). Reflux allows prolonged exposure of digesta to the enzymatic and mechanical systems of the gastrointestinal tract, thus leading to an increase in digestion and absorption time in the upper intestine. Evidence presented in the current study indicates that gizzard bile acid concentrations, and therefore chyme reflux between the gizzard and duodenum, are increased by dietary yarrow in birds fed fat supplemented diets, with the greatest response seen in birds fed diets containing higher levels of saturated fatty acids. Furthermore, there is evidence to suggest (Hetland and Choct, 2003) that the concentration of digestive enzymes in the upper end of the gut may also be elevated by increased reflux. However, in the current study no significant effects of yarrow supplementation on lipase and amylase activity were seen.

The aim of this experiment was to examine whether yarrow supplementation would have any nutritional benefit for broiler diets containing highly saturated fatty acids. From the results of the present study it can be concluded that the growth performance effects seen following yarrow supplementation were influenced to a considerable degree by the type of fat in the diet, with the most profound performance effects observed when diets containing highly saturated fat sources were fed. However, it is clear that these positive increments in growth performance cannot be attributed to increased nutrient availability, as AME and dry matter and fat

digestibility co-efficients were unaffected by yarrow supplementation. Similarly, activity levels of lipase and amylase activity in the small intestine were not affected. However, examination of bile acid concentrations in the gizzard clearly point to another possible mechanism of action for yarrow. Bile acid concentrations in the gizzard are indicative of the degree of gastrointestinal reflux occurring in the small intestine. Elevated gastrointestinal reflux allows more rapid breakdown of food particles. This would facilitate more rapid absorption of nutrients in the proximal small intestine, which would reduce the amount of substrates available for fermentation.

Studies by Daenicke *et al.* (1997b; 2000) have demonstrated that increasing the saturation of diets fed to young broiler chickens results in an increase in intestinal viscosity. Increasing intestinal viscosity slows down the rate of digesta passage (van der Klis *et al.*, 1993), particularly when diets containing saturated fat are fed (Mateos and Sell, 1981). This drastically changes the microbial balance in the gut by decreasing the amount of available oxygen resulting in a stable environment for fermentative bacteria to proliferate (Wagner and Thomas, 1978). Studies by Choct *et al.* (1999, 2000) have demonstrated, through measurement of ileal VFA production, that reducing hind gut fermentation leads to improvements in growth performance. It would appear from the results of the current study that yarrow, when fed in conjunction with highly saturated fat sources, may ameliorate the detrimental effects of fermentation. It seems that this is achieved through increased gastrointestinal reflux, which increases mechanical degradation of feed particles thus enabling more rapid assimilation of nutrients. As a result of this, higher proportions of major nutrients are digested earlier in the proximal end of the tract, which reduces the amount of fermentable substrates available to the hind gut microflora. The hormone CCK mediates control of gastrointestinal reflux via the vagal system. Studies in rats (Lu *et al.*, 2003) have demonstrated

that CCK responsive cells are sensitive to bitter stimuli (quinine and/or caffeine), and elevated circulating CCK is seen in response to feeding these compounds. Historically, it has been conjectured that the bitter principles present in yarrow exert their digestive enhancing effects via stimulation of the vagus nerve (Moerman, 1977; Hoffman, 1998; Saller *et al.*, 2001). The results of the current study support this hypothesis.

4. GENERAL DISCUSSION

As a result of the imminent ban on dietary antimicrobial growth promoters in the EU, interest in alternative options has arisen in order for producers to remain competitive. One alternative area currently being investigated concerns the use of herbs and spices for growth promotion. Herbs and spices have been exploited by man over millennia, offering a wide range of pharmacological benefits including antimicrobial, immunity boosting and digestion enhancing properties. In addition, botanical products are regarded as being 'natural' and thus are perceived in a positive manner by consumers, which should not be underestimated with the current consumer concerns over agricultural production methods and food safety. The review of available literature documenting the effects of herbs and spices on growth performance in non-ruminants, which is scarce, indicates that these products can be beneficial in broiler production, but that growth response is highly variable. Factors affecting the degree of response include rearing environment and diet quality, which is consistent with other growth promoting substances, including AGP.

The literature review indicated that in-depth research is lacking in this area: much of the published work is 'production' orientated with little emphasis on the chemical composition of the plant products or the mechanisms of action *in vivo*. The review also recognised that the chemical composition of herbs and spices is inherently variable, and that they contain many active components which may be responsible for the positive effects seen *in vivo*.

Initial 'screening' experiments carried out on the six selected botanical products demonstrated that both garlic powder and yarrow supplementation resulted in improved FCE during the grower production phase. However, improvements were small, which may have been due to

the clean environmental conditions and highly digestible diet offered, an explanation proposed by others working in this field (Cross *et al.*, 2002; Lee *et al.*, 2003). It was postulated that these improvements were as a result of the antimicrobial properties of the two plants documented *in vitro*.

The next phase of experiments were designed to assess the effects of garlic powder and yarrow supplementation on the growth performance of broilers reared in conditions that were closer to the commercial situation, and, in the event of a positive growth response, to test that the hypothesis that any growth performance improvements seen were as a result of the reported *in vitro* antimicrobial activity of the two botanical products. In the garlic experiment, there was no evidence to suggest that garlic supplementation exerted any positive growth performance effects, even though the same garlic product was included at the inclusion levels used previously, and the general bird growth performance was below Ross targets. Ergo, caecal microbiology was not performed on samples taken from these birds, and the investigation of garlic as a natural growth promotant was not pursued.

It is not clear why the response to garlic was so variable: the garlic product used and inclusion rate were consistent for all three experiments, yet a positive growth response was only observed in one of the three experiments where garlic was fed. Numerous other groups have evaluated the effects of garlic supplementation, with varying growth performance responses. Of the eight experiments published, five documented statistically significant ($P<0.05$) positive growth responses (Qureshi *et al.*, 1983a; Horton *et al.*, 1991b; Mottaghitalab, 2000; Tucker, 2002; Demir *et al.*, 2003) and three did not report a growth response (de Frietas *et al.*, 2001; Al-Homidan, 2004; Cross *et al.*, 2004b). Garlic inclusion rates varied from 1 to 100 g kg^{-1}. The average response to garlic in experiments where a positive growth response was detected

was a 9% increase in weight gain relative to controls. It is not clear why the response to garlic is so variable; inclusion levels as low as 1g kg^{-1} have shown positive effects in one experiment (Horton *et al.*, 1991b) and yet not in another (Cross *et al.*, 2004b). Despite several reported positive growth responses following garlic supplementation, no mode of action has been satisfactorily proven, although the mechanism is widely speculated to be connected to the antimicrobial effects of garlic. In this series, only one of the three experiments yielded a statistically significant growth improvement. Results from the two screening experiments indicated that birds failed to show a growth response to either organic acids or oregano, both of which have been well researched as alternative growth promoters with antimicrobial properties (Patten and Waldroup, 1988; Skinner *et al.*, 1991; Waldenstedt, 2000; Demir *et al.*, 2003). It may be that the positive effects of garlic on FCE during the second screening experiment were not as a result of antimicrobial activity *in vivo*.

The findings of the yarrow floor-pen experiment indicated that dietary yarrow exerts consistent positive growth performance effects, but that no effects on caecal microflora populations were noted. Yarrow has proven antibacterial action *in vitro* (Bishop and MacDonald, 1951; Candan *et al.*, 2003), and GC-MS analysis of essential oil distilled from the yarrow material used in these experiments revealed that it comprises predominantly terpenes, both mono- and sesqui-, which are known for their antibacterial activity (Harborne *et al.*, 1999). However, in agreement with the work of Cross *et al.* (2001), it appears that the mechanism for yarrow is not connected to its antimicrobial activity *in vitro*. It is possible that the antibacterial compounds in yarrow *in vitro* are not present in sufficient concentrations to exert any antimicrobial effects *in vivo*.

Following the elimination of garlic powder, the main emphasis of the project focussed on elucidating the mechanisms of action for yarrow. As previously demonstrated, yarrow does not have any antimicrobial effects *in vivo*. A review of the literature pertaining to yarrow indicated that it has been used extensively as an aid to treat gastric problems, and anecdotal evidence states that the bitter components (sesquiterpenes lactones) present in the herb stimulate the secretion and subsequent activity of digestive enzymes. Furthermore, several recent publications in the area of herbal supplementation have suggested that feeding highly digestible basal diets masks the positive effects of herbs *in vivo*, making detection of beneficial growth performance effects more difficult. An experiment was designed to test the hypothesis that yarrow supplementation would improve growth performance and increase digestive enzyme activity, and that the effects would be more prominent in birds fed low nutrient density diets. Contrary to expectations, no statistical improvements in growth performance were noted, either in the birds fed highly digestible or high fibre diets. However, there was an interesting interaction between basal diet and yarrow supplementation on AME whereby yarrow increased AME by approximately 0.5 MJ kg DM^{-1} ($P<0.05$) in birds fed highly digestible diets. This corresponded to a similar interaction observed on lipase activity at 18 days of age: yarrow supplementation increased lipase activity by some 20% in birds fed highly digestible diets ($P<0.05$), but not in birds fed high fibre diets. It was conjectured that yarrow supplementation of highly digestible diets results in improved fat digestion, specifically at a time when fat digestion is crucial, that is, in the young broiler.

The null hypothesis for the final experiment was that yarrow supplementation would improve fat digestibility in growing broilers, and that the effect would be greater when highly saturated fats were fed. Indeed, yarrow supplementation was found to have important growth effects, predominantly in birds fed highly saturated diets. However, yarrow did not appear to affect

either nutrient digestibility or lipase activity in the small intestinal chyme, as it did in the previous experiment. This may have been because of inherent differences in diet formulation between the two experiments: in experiment five, diets were formulated to have 25g kg^{-1} added fat, whereas in experiment 6 supplementary fat was included at 50g kg^{-1}. Despite finding no statistical effects on lipase activity or AME, an interaction between yarrow supplementation and saturation of the diet was observed in bile acid concentrations; gizzard bile acid concentrations were elevated in birds fed saturated diets, but relatively stable in birds fed less saturated diets. Increased bile acid concentrations are indicative of the extent of gastro-intestinal reflux, and results of this experiment suggest that the positive increments in growth performance observed in birds fed highly saturated diets may be as a result of improved efficiency of digestion in the proximal small intestine. This is likely to reduce the amount of substrates available for bacterial fermentation further down the gut. In addition, the increased mechanical breakdown of feed particles and subsequent absorption of nutrients may increase the rate of passage, allowing birds to consume more feed and thus improve growth and efficiency parameters.

In summary, it can be concluded that yarrow supplementation of broiler chickens can have a positive effect on growth performance, but that the responses are variable (table 4.1). Of the experiments where a positive growth response was observed, the average improvement of birds fed yarrow supplemented diets relative to those fed control diets were +12% daily weight gain and +15% FCE, with an overall reduction in feed intake of 3.5%. When collating similar data from the experiments of Cross *et al.* (2002; 2004b), improvements of 9, 3 and 4% for weight gain, FCE and feed intake respectively following yarrow herb supplementation can be calculated. It would appear that the magnitude of response to yarrow supplementation was higher in the current series of experiments. This may be attributed to the basal diets fed: in the

current series, no response to yarrow was observed in birds fed diets of low nutrient density, and the positive *in vivo* effects of yarrow supplementation on AME and lipase activity were only observed in high energy density diets. The basal diets used by Cross *et al.* were consistently lower in energy density than those fed in the current series (with the exception of those formulated to be low energy density), which may explain the lesser growth performance effects observed following yarrow supplementation.

Table 4.1 Positive effects observed *in vivo* with dietary yarrow supplementation

Experimental conditions	**Basal diet**	**Bird age (days)**	**Positive effect observed**	***P***	**Change relative to controls**	**Reference**
Cages	Highly digestible	17-27	Improved FCE	0.021	+ 12.9%	Section 3.1.8; Table 3.1.11
Floor pens	Highly digestible	0-18	Birds ate less feed, but attained similar weight gains and FCE to controls	0.097	- 3.3%	Section 3.2.7; Table 3.2.11
Cages	Highly digestible	0-36			- 7.8%	Section 3.3.4; Table 3.3.6
	High Fibre/low energy			0.020	- 6.2%	
Cages	Highly digestible	24-27	Improved AME	0.019	+ 3.7%	Section 3.3.4; Table 3.3.9
Cages	Highly digestible	18	Higher lipase activity in small intestinal chyme	0.018	+ 20.8%	Section 3.3.4; Table 3.3.10
Cages	Highly saturated	10-21	↑ DLWG ↑ DFI ↑ FCE	0.002 0.036 0.002	+ 25.9% + 6.9% + 17.9%	Section 3.4.4; Table 3.4.4
Cages	Highly saturated	10-21	Elevated gizzard bile acid concentrations	0.006	+ 163%	Section 3.4.4; Table 3.4.7

The final experiment led to the discovery that yarrow supplementation increases bile acid concentrations in the gizzard, which may indicate increased gastro-intestinal reflux and subsequent improvements in the efficiency of digestion. Although this effect was observed predominantly in birds fed diets containing high amounts of saturated fatty acids, there was a moderate effect (+3.3%) in birds fed diets containing soya bean oil. It may be that the smaller performance effects observed throughout this series of experiments (table 4.1) were as a result of increased gastro-intestinal reflux and the ensuing improvements in nutrient absorption.

As indicated in the literature review, herb composition is notoriously variable, with many factors affecting which compounds are present in the final herbal product. The compositional analysis of the yarrow used in the current experiments was consistent with reported values in the literature (Rohloff *et al.*, 2000; Candan *et al.*, 2003; Cross, 2004). The essential oil distilled from the herb material used in the feeding experiments was rich in terpenes, mainly mono- and sesqui-terpenes, which possess antimicrobial and 'bitter' properties (Harborne *et al.*, 1999). Although yarrow has been shown to exert antimicrobial properties *in vitro* (Bishop and MacDonald, 1951; Candan *et al.*, 2003), it seems to be insufficient to have an effect *in vivo*; but sesquiterpenes are also known for their bitter properties (Rodriguez *et al.*, 1976; Picman, 1986), which are thought to increase digestive enzyme activity and improve digestion (Chandler, 1989; Hoffman, 1998; Gill, 1999; McCartney, 2002). However, despite an extensive search of herbal chemistry texts and the available literature pertaining to the chemical composition of yarrow, little information was found concerning the components identified and the pharmacological actions or biochemical pathways that they may affect *in vivo*. Compositional analysis of yarrow could only be carried out on the essential oil extracted from the herb. The chemical composition of essential oils is not identical to that of the herb from which the oil is extracted (Chevallier, 1986). Indeed, a study by Cross *et al.* (2002)

demonstrated large discrepancies between feeding yarrow as a herb and an essential oil: birds fed yarrow herb had higher growth rates (+21%; $P<0.001$), higher feed intakes (+12%; $P<0.05$) and better FCE (+7 points; $P<0.05$) than their oil fed conspecifics, indicating some differences between the two yarrow forms. However, compositional analysis can only be performed on the essential oil, so this was the only available option. It seems likely that there is a compound in the herb that is not present or active in the essential oil which is responsible for the beneficial effects observed *in vivo*. However, nothing in the literature could be found to corroborate this.

The three phases of the present study comprised identification of potentially beneficial botanical products, evaluation of growth performance effects and investigation into the likely mechanism(s) of action. The next phase of this work would be to identify scenarios where yarrow supplementation could be utilised for commercial gain. The magnitude of response to yarrow appears to be dependant on the quality of the basal diet. The last experiment in the series demonstrated that the greatest responses to yarrow supplementation were observed when diets containing highly saturated fats were fed. However, it is likely that the positive effect of yarrow on growth performance would diminish with increasing age, as birds are able to adapt to highly saturated diets as their gut develops (Krogdahl and Sell, 1989; Noy and Sklan, 1995). During the experiment, improvements in FCE of approximately 10 points were observed from 10-21 days of age; so assuming a conservative estimate of a 5 point improvement in FCE throughout the growing period, yarrow supplementation would realise an additional 50kg of broiler meat per tonne of feed. Preliminary calculations indicate that addition of the yarrow product would increase feed costs by approximately £2 $tonne^{-1}$, which, assuming a price of 50p kg liveweight of broiler, gives a return on investment of 12 to 1. However, this is only applicable when highly saturated diets are fed.

Further work that gives a greater understanding of the interactions between yarrow, diet saturation and growing period may enable the prediction of exact scenarios where yarrow supplementation would be financially viable in commercial broiler production. It would also be of interest to quantify the effects of yarrow supplementation on ileal fat and dry matter digestibility in birds fed diets containing higher levels of saturated fat. This may clarify speculation that increased gastrointestinal reflux improves digestibility at the proximal end of the small intestine, reducing the amount of substrate available for fermentation in the distal intestine. In order to explain the mechanism by which the 'bitter' sesquiterpenes exert their effects, it would be of interest to quantify the effects of yarrow supplementation on CCK activity. CCK stimulates gastrointestinal reflux, as indicated by bile acid concentration in the gizzard, and stimulates the release of digestive enzymes (Denbow, 2000). However, measurement of lipase activity in the small intestinal chyme is highly variable, which makes detection of statistical differences difficult. Measuring CCK activity directly may offer a less variable parameter by which to assess the effects of yarrow. In addition, it may be of value to investigate the effects of yarrow on gizzard development, which is thought to be stimulated by increasing gastrointestinal reflux (Hetland and Choct, 2004).

Ultimately, the direct application of a method whereby an ideal amount of yarrow is premixed into highly saturated fat sources prior to dietary incorporation may be of commercial value. In order to determine the 'ideal' amount of yarrow needed, dose response experiments which take into account different fat types and their optimum yarrow supplementation rates may be of value.

5. CONCLUSIONS

- Two 'screening' experiments were carried out to examine the effects of six chosen botanical products on the growth performance of caged broilers.
- In the first screening experiment, no statistically significant effects on growth performance were observed.
- In the second screening experiment, both garlic powder and yarrow improved growth performance.
- One further experiment examining the effect of garlic on growth performance revealed no positive growth performance effects in floor reared broilers. Thus one experiment indicated a positive growth effect of garlic powder but two did not.
- Yarrow supplementation was found to be beneficial to broiler growth performance in both caged and floor-rearing conditions.
- Yarrow supplementation did not affect gut microbial populations and thus the yarrow used in the present series of experiments is not considered to have antimicrobial properties *in vivo*.
- The positive effect of yarrow supplementation and its interaction with bird age seem to be dependant on diet composition.
- The most profound effects following yarrow supplementation were seen when highly saturated diets were offered to young broilers.
- Yarrow supplementation increased lipase activity ($P<0.05$) in small intestinal chyme in birds fed high nutrient dense diets, but not in birds fed low nutrient dense diets.
- Yarrow supplementation increased AME ($P<0.05$) in birds fed high nutrient density diets, but not in birds fed low nutrient dense diets.

- Yarrow supplementation of diets containing highly saturated fat sources increased gizzard bile acid concentrations ($P<0.05$), which are indicative of the extent of gastro-intestinal reflux.
- Yarrow supplementation of diets containing palm oil fatty distillate increased bird growth performance to a level equal to that of birds fed diets containing soya bean oil.
- Yarrow supplementation may increase gastro-intestinal reflux, which may improve efficiency of digestion in the proximal intestine and thus reduce the amount of substrate available for fermentation in the lower intestine. The 'bitter' compounds (sesquiterpene lactones such as cadinene) found in yarrow may stimulate CCK release, which in turn stimulates the activity of digestive enzymes and gastro-intestinal reflux.
- Potentially, yarrow may be used commercially to improve the utilisation of highly saturated dietary fats.
- Further investigation into the interactions between yarrow supplementation, feeding period and dietary saturation are needed in order to identify optimal situations for yarrow usage.

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